



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> C07K 14/715, 16/24, C07H 21/04, C12N 15/12, 15/63	<b>A1</b>	<b>(11) International Publication Number:</b> WO 95/30695 <b>(43) International Publication Date:</b> 16 November 1995 (16.11.95)
<b>(21) International Application Number:</b> PCT/US95/05585 <b>(22) International Filing Date:</b> 4 May 1995 (04.05.95)  <b>(30) Priority Data:</b> 08/236,919 6 May 1994 (06.05.94) US 08/300,903 6 September 1994 (06.09.94) US  <b>(71) Applicant:</b> IMMUNEX CORPORATION [US/US]; 51 University Street, Seattle, WA 98101 (US).  <b>(72) Inventors:</b> ANDERSON, Dirk, M.; 3616 N.W. 64th Street, Seattle, WA 98107 (US). GIRI, Judith, G.; 8020 Corliss Avenue North, Seattle, WA 98103 (US).  <b>(74) Agent:</b> PERKINS, Patricia, Anne; Immunex Corporation, 51 University Street, Seattle, WA 98101 (US).		<b>(81) Designated States:</b> AU, CA, JP, MX, NZ, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> INTERLEUKIN-15 RECEPTORS  <b>(57) Abstract</b>  There are disclosed Interleukin-15 Receptor (IL-15R) proteins, DNAs and expression vectors encoding IL-15R, and processes for producing IL-15R as products of recombinant cell cultures.		

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**TITLE**

## Interleukin-15 Receptors

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**BACKGROUND OF THE INVENTION**

The present invention relates generally to cytokine receptors, and more specifically, to Interleukin-15 receptors.

Interleukin-15 (IL-15) is a recently identified cytokine with biological activities similar to IL-2 (Grabstein et al., *Science* 264:965, 1994). There is approximately 96%  
10 nucleotide sequence identity and 96% amino acid sequence identity between human and simian IL-15, and approximately 81% nucleotide sequence identity and 73% amino acid sequence identity between human and murine IL-15.

Northern analysis of a variety of human tissues indicated that IL-15 mRNA is expressed by many human tissues and abundantly by placenta and skeletal muscle.  
15 Significant levels of IL-15 mRNA were also observed in other tissues including kidney, lung, liver, and heart. The best sources of IL-15 mRNA so far observed have been adherent mononuclear cells (monocyte enriched, PBM) and epithelial and fibroblast cell lines such as CV-1/EBNA and IMTLH. Activated peripheral blood T cells (PBT), a rich source of IL-2, express no detectable IL-15 mRNA.

20 IL-15 shares many biological properties with Interleukin-2 ("IL-2"). These properties include proliferation and activation of human and murine T cells and the generation of lymphokine activated killer cells (LAK), natural killer cells (NK) and cytotoxic T lymphocytes (CTL). IL-15 also can co-stimulate with CD40 ligand (CD40L) proliferation and immunoglobulin secretion by B lymphocytes.

25 In view of the shared biological properties with IL-2, tests were conducted to determine whether IL-15 uses any of the components of the IL-2 receptor. IL-2 cell surface receptors (IL-2R) contain at least three subunits,  $\alpha$ ,  $\beta$  and  $\gamma$  (Toshikazu et al., *Science*, 257: 379 (1992); see also Minami et al., *Annu. Rev. Immunol.* 11,245, 1993, for a recent review). The  $\beta$  and  $\gamma$  chains are required for high affinity IL-2 binding and IL-2  
30 signaling and are members of the hematopoietin receptor superfamily. The  $\alpha$  chain (or p55) is a low affinity, non-signaling binding subunit, and the only cytokine receptor member of a large family of binding proteins whose members include complement receptor proteins (Perkins et al., *Biochemistry* 27:4004, 1988; Davie et al., *Cold Spring Harb. Symp. Quant. Biol.* 51:509, 1986). The  $\gamma$  chain of the IL-2R has been shown recently to  
35 be shared by receptors for several other cytokines (IL-4, IL-7, IL-9; (Noguchi et al., *Science* 262:1877, 1993; Kondo, et al., *Science* 262:1874, 1993; Kondo et al., *Science* 263:1453, 1994; Russell et al., *Science* 262:1880, 1993; Russell, et al., *Science* 266:1042, 1994) and designated the common  $\gamma$  chain or  $\gamma_c$ .

Several lines of evidence suggest that there is an IL-15 specific binding protein. For example, an IL-3 dependent murine cell line, 32D (J. S. Greenberger et al., *Fed. Proc.* 42: 2762 (1983)), expressed the complete IL-2R and proliferated in response to IL-2, but cannot bind or respond to IL-15 (Grabstein et al., *supra*). Similarly, early murine pre-T  
5 cells derived from day 13 fetal liver that lack CD3, CD4 and CD8 expression (triple negative, or TN, cells) expressed all three IL-2R subunits, proliferated in response to IL-2, but did not bind or respond to IL-15 (Giri et al., *EMBO J.* 13:2822, 1994). On the other hand, certain human cell types and cell lines (e.g., umbilical vein endothelial cells, fibroblasts and thymic and stromal cells) did not bind IL-2 but bound IL-15 with high  
10 affinity (Giri et al., *supra*).

Additionally, antibodies directed against the  $\alpha$  chain of the IL-2 receptor (anti-IL-2R $\alpha$ ) have no effect on IL-15 (Grabstein et al., *supra*; Giri et al., *supra*). Antibodies directed against the IL-2R $\beta$ , however, are able to block the activity of IL-15, suggesting that IL-15 uses the  $\beta$  chain of IL-2R. Similarly, some cells require the  $\gamma$  chain of IL-2R for  
15 IL-15 signal transduction (Giri et al., *supra*) IL-15 requires the  $\beta$  chain of the IL-2R for all the biological activities tested, but the  $\alpha$  chain of the IL-2R is not required (Giri et al., *supra*; Grabstein et al., *supra*). However, prior to the present invention, neither an IL-15-specific binding protein, nor a DNA encoding such protein, had been isolated.

## 20 SUMMARY OF THE INVENTION

The present invention provides isolated Interleukin-15 receptor (IL-15R) and isolated DNA sequences encoding IL-15R, in particular, human and murine IL-15R, or analogs thereof. Preferably, such isolated DNA sequences are selected from the group consisting of (a) DNA sequences comprising a nucleotide sequence derived from the  
25 coding region of a native IL-15R gene; (b) DNA sequences capable of hybridization to a DNA of (a) under moderate to high stringency conditions and that encode biologically active IL-15R; and (c) DNA sequences that are degenerate as a result of the genetic code to a DNA sequence defined in (a) or (b) and that encode biologically active IL-15R. The present invention also provides recombinant expression vectors or plasmids and  
30 transformed host cells comprising the DNA sequences defined above, recombinant IL-15R proteins produced using the recombinant expression vectors, plasmids or transformed host cells, and processes for producing the recombinant IL-15R proteins utilizing the expression vectors, plasmids or transformed host cells.

The present invention also provides substantially homogeneous preparations of IL-  
35 15R protein. The present invention also provides compositions for use in assays for IL-15 or IL-15R, purification of IL-15, or in raising antibodies to IL-15R, comprising effective quantities of the IL-15R proteins of the present invention.

These and other aspects of the present invention will become evident upon reference to the following detailed description.

### **BRIEF DESCRIPTION OF THE DRAWINGS**

5        Figure 1 illustrates the inhibition of binding of radiolabeled IL-15 to CTLL.2 cells by soluble murine IL-15 receptor (HIS-IL15R).

Figure 2 presents a sequence alignment between the murine IL-15 receptor and the human IL15 receptor (clone W5). The top line represents the amino acid sequence of human IL-15R; the bottom line represents the amino acid sequence of murine IL-15R. The  
10        amino acid sequence has been separated into several protein domains:

1. signal sequence
2. structural domain 1
3. proline-rich, flexible hinge region
4. structural domain 2
- 15        5. transmembrane domain
6. cytoplasmic domain

The primary amino acid sequence was also analyzed for predicted structural characteristics, and found to share common features with a group of complement factors, and the  $\alpha$  subunit of IL-2 receptor. Certain structural characteristics of the IL-15R are also designated in  
20        Figure 2:

- $\beta$ : beta sheet
- L: loop
- bold**: amino acids conserved among IL-15R and related proteins (i.e., complement control proteins, IL-2 receptor  $\alpha$  chain)
- 25        shaded: putative IL-15 binding region

### **DETAILED DESCRIPTION OF THE INVENTION**

"Interleukin-15 receptor," "IL-15R" and "IL-15R $\alpha$ " refer to proteins that are present on many cell types, including cells of lymphoid origin, as well as non-lymphoid  
30        cells such as fresh human endothelial cells, and stromal cells types from bone marrow, fetal liver and thymic epithelium. As used herein, the above terms include analogs or fragments of native and recombinant IL-15R proteins with IL-15-binding activity. Specifically included are truncated, soluble or fusion forms of IL-15R protein as defined below. In the absence of any species designation, IL-15R refers generically to mammalian IL-15R,  
35        including but not limited to, human, murine, and bovine IL-15R. Similarly, in the absence of any specific designation for deletion mutants, the term IL-15R means all forms of IL-15R, including mutants and analogs that possess IL-15R biological activity.

"Soluble IL-15R" or "sIL-15R" as used in the context of the present invention refer to proteins, or substantially equivalent analogs, that are substantially similar to all or part of the extracellular region of a native IL-15R and are secreted by the host cell but retain the ability to bind IL-15. Soluble IL-15R proteins may also include part of the transmembrane region or part of the cytoplasmic domain or other sequences, provided that the soluble IL-15R proteins are capable of being secreted from the host cell in which they are produced.

The term "isolated" or "purified", as used in the context of this specification to define the purity of IL-15R protein or protein compositions, means that the protein or protein composition is substantially free of other proteins of natural or endogenous origin and contains less than about 1% by mass of protein contaminants residual of production processes. Such compositions, however, can contain other proteins added as stabilizers, carriers, excipients or co-therapeutics. IL-15R is purified to substantial homogeneity if no other proteins of natural or endogenous origin, or protein contaminants residual of production processes, are detected in a polyacrylamide gel by silver staining.

The term "substantially similar," when used to define either amino acid or nucleic acid sequences, means that a particular subject sequence, for example, a mutant sequence, varies from a reference sequence (e.g., a native sequence) by one or more substitutions, deletions, or additions, the net effect of which is to retain biological activity of the IL-15R protein as may be determined, for example, in IL-15R binding assays, such as is described in Example 1 below. Substantially similar analog protein will be greater than about 30 percent similar to the corresponding sequence of the native IL-15R. More preferably, the analog proteins will be greater than about 80 percent identical to the corresponding sequence of the native IL-15R. For fragments of IL-15R proteins, (e.g., soluble IL-15R polypeptides), the term "80 percent identical" refers to that portion of the reference native sequence that is found in the IL-15R fragment.

Computer programs are available for determining the percent identity between two DNA or amino acid sequences (e.g., between a mutant sequence and a native sequence). One example is the GAP computer program, version 6.0, described by Devereux et al., *Nucl. Acids Res.* 12:387 (1984) and available from the University of Wisconsin Genetics Computer Group (UWGCG). The GAP program uses the alignment method of Needleman and Wunsch, *J. Mol. Biol.* 48:443 (1970), as revised by Smith and Waterman, *Adv. Appl. Math* 2:482 (1981).

Alternatively, nucleic acid subunits and analogs are "substantially similar" to the specific native DNA sequences disclosed herein if (a) the DNA sequence is derived from the coding region of a native mammalian IL-15R gene; (b) the DNA sequence is capable of hybridization to a native IL-15R DNA sequence under moderately stringent conditions (i.e., 50°C, 2x SSC) and encodes biologically active IL-15R protein; or (c) the DNA sequence is degenerate as a result of the genetic code to one of the foregoing native or

hybridizing DNA sequences and encodes a biologically active IL-15R protein. DNA sequences that hybridize to a native IL-15R DNA sequence under conditions of high stringency, and that encode biologically active IL-15R, are also encompassed by the present invention. Moderate and high stringency hybridization conditions are terms  
5 understood by the skilled artisan and have been described in, for example, Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2 ed. Vol. 1, pp. 1.101-104, Cold Spring Harbor Laboratory Press (1989). IL-15R proteins encoded by the foregoing DNA sequences are provided by the present invention.

"Recombinant DNA technology" or "recombinant", as used herein, refers to  
10 techniques and processes for producing specific polypeptides from microbial (e.g., bacterial, fungal or yeast) or mammalian cells or organisms (e.g., transgenics) that have been transformed or transfected with cloned or synthetic DNA sequences to enable biosynthesis of heterologous peptides. Native glycosylation patterns will only be achieved with mammalian cell expression systems. Yeast provide a distinctive glycosylation pattern.  
15 Prokaryotic cell expression (e.g., *E. coli*) will generally produce polypeptides without glycosylation.

"Biologically active", as used throughout the specification as a characteristic of IL-15R, means that a particular molecule shares sufficient amino acid sequence similarity with a native IL-15R protein to be capable of binding detectable quantities of IL-15, preferably  
20 with affinity similar to native IL-15R.

A "DNA sequence" refers to a DNA polymer, in the form of a separate fragment or as a component of a larger DNA construct, that has been derived from DNA isolated at least once in substantially pure form (i.e., free of contaminating endogenous materials) and in a quantity or concentration enabling identification, manipulation, and recovery of its  
25 component nucleotide sequences by standard biochemical methods such as those outlined in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989). Such sequences are preferably provided in the form of an open reading frame uninterrupted by internal nontranslated sequences, or introns, that are typically present in eukaryotic genes. Sequences of non-  
30 translated DNA may be present 5' or 3' from an open reading frame, where the same do not interfere with manipulation or expression of the coding regions.

"Nucleotide sequence" refers to a heteropolymer of deoxyribonucleotides. DNA sequences encoding the proteins provided by this invention may be assembled from cDNA fragments and short oligonucleotide linkers, or from a series of oligonucleotides, to  
35 provide a synthetic gene that is capable of being expressed in a recombinant transcriptional unit.

"Recombinant expression vector" refers to a plasmid comprising a transcriptional unit comprising an assembly of (1) a genetic element or elements having a regulatory role in

gene expression, for example, promoters or enhancers, (2) a structural or coding sequence that is transcribed into mRNA and translated into protein, and (3) appropriate transcription and translation initiation and termination sequences. Structural elements intended for use in yeast expression systems preferably include a leader sequence enabling extracellular  
5 secretion of translated protein by a host cell. Alternatively, where recombinant protein is expressed without a leader or transport sequence, it may include an N-terminal methionine residue. This residue may optionally be subsequently cleaved from the expressed recombinant protein to provide a final product.

"Recombinant microbial expression system" means a substantially homogeneous  
10 monoculture of suitable host microorganisms, for example, bacteria such as *E. coli* or yeast such as *S. cerevisiae*, that has stably integrated a recombinant transcriptional unit into chromosomal DNA or carries the recombinant transcriptional unit as a component of a resident plasmid. Generally, cells constituting the system are the progeny of a single ancestral transformant. Recombinant expression systems as defined herein will express  
15 heterologous protein upon induction of the regulatory elements linked to the DNA sequence or synthetic gene to be expressed.

#### Isolation of DNA Encoding IL-15R

As shown by Scatchard analysis of iodinated IL-15 binding, activated PBT as well  
20 as antigen specific T cell clones express only a few hundred receptors for IL-15. Cells from the murine Th2 CD4<sup>+</sup> cell clone, D10 (Kaye et al., *J. Immunol.* 133:1339 (1984)), express up to 24,000 IL-15 receptors when cultured with IL-2. A murine DNA sequence encoding murine IL-15R was isolated from a cDNA library prepared using standard methods by reverse transcription of polyadenylated RNA isolated from D10 cells.  
25 Transfectants expressing biologically active IL-15R were initially identified using a slide autoradiographic technique, substantially as described by Gearing et al., *EMBO J.* 8:3667 (1989).

A D10 cDNA library in plasmid pDC304 was prepared as described in Larsen et al., *J. Exp. Med.*, 172:159 (1990). pDC304 is derived from pDC302 previously described  
30 by Mosley et al., *Cell*, 59: 335-348 (1989) by deleting the adenovirus tripartite leader (TPL) in pDC302.

Using this approach, approximately 20,000 cDNAs were screened in pools of approximately 1000 cDNAs each using the slide autoradiographic method until assay of one transfectant pool showed multiple cells clearly positive for IL-15 binding. This pool  
35 was then partitioned into pools of approximately 100 and again screened by slide autoradiography and a positive pool was identified. Individual colonies from this pool of approximately 100 were screened until a single clone (clone D1-4-D5) was identified that directed synthesis of a surface protein with detectable IL-15 binding activity. This clone



was isolated and sequenced to determine the sequence of the murine IL-15R cDNA clone, D1-4-D5. The cloning vector pDC304 containing the murine IL-15R cDNA clone, D1-4-D5, was deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852 USA ("ATCC") in accordance with the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure on April 22, 1994, under accession number ATCC 62659. The deposit was named "D1-4-D5 (pDC304:muIL-15R)" and comprised an *E. coli* strain containing a murine IL-15R cDNA insert that is made up of a 71-bp 5' noncoding region preceding an open reading frame of 792 bp and a 995-bp 3' non coding region (the 3'-most approximately 200 bp of which is likely to be derived from non-related sequence). The nucleotide sequence of the open reading frame is disclosed in SEQ ID NO:1. All restrictions on the availability to the public of the material deposited will be irrevocably removed upon the granting of a patent.

A probe may be constructed from the murine sequence and used to screen various other mammalian cDNA libraries. cDNA clones that hybridize to the murine probe are then isolated and sequenced.

Like most mammalian genes, mammalian IL-15R is encoded by a multi-exon gene. IL-15R variants can be attributed to different mRNA splicing events following transcription or from proteolytic cleavage of the IL-15R protein, wherein the IL-15R binding property is retained. Alternative splicing of mRNA may yield a truncated but biologically active IL-15R protein, such as a soluble form of the protein. Variations attributable to proteolysis include, for example, differences in the N- or C-termini upon expression in different types of host cells, due to proteolytic removal of one or more terminal amino acids from the IL-15R protein (generally from 1-5 terminal amino acids). Signal peptides may be cleaved at different positions in a given protein, resulting in variations of the N-terminal amino acid of the mature protein. These IL-15R variants share large regions of identity or similarity with the cDNAs claimed herein and are considered to be within the scope of the present invention.

### 30 Proteins and Analogs

The present invention provides recombinant mammalian IL-15R polypeptides. Isolated IL-15R polypeptides of this invention are substantially free of other contaminating materials of natural or endogenous origin and contain less than about 1% by mass of protein contaminants residual of production processes. The IL-15R polypeptides of this invention are optionally without associated native-pattern glycosylation.

Mammalian IL-15R of the present invention includes, by way of example, primate, human, murine, canine, feline, bovine, ovine, equine and porcine IL-15R. The amino acid sequence of a full length murine IL-15R (i.e., including signal peptide, extracellular

domain, transmembrane region and cytoplasmic domain) is shown in SEQ ID NOs:1 and 2. The amino acid sequence in SEQ ID NOs:1 and 2 predicts a type 1 membrane protein (i.e., a single transmembrane region with a N-terminal extracellular domain and a C-terminal cytoplasmic domain). The predicted signal peptide cleavage occurs between amino acids 30 and 31 in SEQ ID NO:2. The predicted transmembrane region includes amino acids 206 to 226 in SEQ ID NO:2. Mammalian IL-15R cDNA can be obtained by cross species hybridization, for example, by using a single stranded probe derived from the murine IL-15R DNA sequence, SEQ ID NO:1, as a hybridization probe to isolate IL-15R cDNAs from mammalian cDNA libraries. The isolated IL-15R cDNAs then can be transfected into expression vectors and host cells to express the IL-15R proteins.

Derivatives of IL-15R within the scope of the invention also include various structural forms of the primary protein that retain biological activity. Due to the presence of ionizable amino and carboxyl groups, for example, an IL-15R protein may be in the form of acidic or basic salts, or may be in neutral form. Individual amino acid residues may also be modified by oxidation or reduction.

The primary amino acid structure may be modified by forming covalent or aggregative conjugates with other chemical moieties, such as glycosyl groups, lipids, phosphate, acetyl groups and the like, or by creating amino acid sequence mutants. Covalent derivatives are prepared by linking particular functional groups to IL-15R amino acid side chains or at the N- or C-termini. Other derivatives of IL-15R within the scope of this invention include covalent or aggregative conjugates of IL-15R or its fragments with other proteins or polypeptides, such as by synthesis in recombinant culture as N-terminal or C-terminal fusions.

When initially expressed in a recombinant system, IL-15R may comprise a signal or leader polypeptide sequence (native or heterologous) at the N-terminal region of the protein. The signal or leader peptide co-translationally or post-translationally directs transfer of the protein from its site of synthesis to its site of function outside the cell membrane or wall, and is cleaved from the mature protein during the secretion process. Further, using conventional techniques, IL-15R polypeptides can be expressed as polypeptide fusions comprising additional polypeptide sequences, such as Fc or other immunoglobulin sequences, linker sequences, or other sequences that facilitate purification and identification of IL-15R polypeptides.

IL-15R derivatives may also be used as immunogens, reagents in receptor-based immunoassays, or as binding agents for affinity purification procedures of IL-15 or other binding ligands. IL-15R derivatives may also be obtained by cross-linking agents, such as m-maleimidobenzoyl succinimide ester and N-hydroxysuccinimide, at cysteine and lysine residues. IL-15R proteins may also be covalently bound through reactive side groups to various insoluble substrates, such as cyanogen bromide-activated, bisoxirane-activated,

carbonyldiimidazole-activated or tosyl-activated agarose structures, or by adsorbing to polyolefin surfaces (with or without glutaraldehyde cross-linking). Once bound to a substrate, IL-15R may be used to selectively bind (for purposes of assay or purification) anti-IL-15R antibodies or IL-15.

5           The IL-15R proteins of the present invention encompass proteins having amino acid sequences that vary from those of native IL-15R proteins, but that retain the ability to bind IL-15. Such variant proteins comprise one or more additions, deletions, or substitutions of amino acids when compared to a native sequence, but exhibit biological activity that is essentially equivalent to that of native IL-15R protein. Likewise, the IL-15R-encoding  
10 DNA sequences of the present invention encompass sequences that comprise one or more additions, deletions, or substitutions of nucleotides when compared to a native IL-15R DNA sequence, but that encode an IL-15R protein that is essentially bioequivalent to a native IL-15R protein. Examples of such variant amino acid and DNA sequences (the "substantially similar" sequences discussed above) include, but are not limited to, the  
15 following.

          Bioequivalent analogs of IL-15R proteins may be constructed by, for example, making various substitutions of residues or sequences or deleting terminal or internal residues or sequences not needed for biological activity. For example, cysteine residues not essential for biological activity can be deleted or replaced with other amino acids to  
20 prevent formation of unnecessary or incorrect intramolecular disulfide bridges upon renaturation.

          Another embodiment of the present invention involves modification of adjacent dibasic amino acid residues to enhance expression of IL-15R in yeast systems in which KEX2 protease activity is present. Generally, substitutions should be made conservatively;  
25 i. e., the most preferred substitute amino acids are those having physiochemical characteristics resembling those of the residue to be replaced. Similarly, when a deletion or insertion strategy is adopted, the potential effect of the deletion or insertion on biological activity should be considered.

          Substantially similar polypeptide sequences, as defined above, generally comprise a  
30 like number of amino acid sequences, although C-terminal truncations for the purpose of constructing soluble IL-15Rs will contain fewer amino acid sequences. In order to preserve the biological activity of IL-15Rs, deletions and substitutions will preferably result in homologous or conservatively substituted sequences, meaning that a given residue is replaced by a biologically similar residue. Examples of conservative substitutions include  
35 substitution of one aliphatic residue for another, such as Ile, Val, Leu, or Ala for one another, or substitutions of one polar residue for another, such as between Lys and Arg; Glu and Asp; or Gln and Asn. Other such conservative substitutions, for example, substitutions of entire regions having similar hydrophobicity characteristics, are well

known. Moreover, particular amino acid differences between human, murine and other mammalian IL-15Rs are suggestive of additional conservative substitutions that may be made without altering the essential biological characteristics of IL-15R.

5 The present invention includes IL-15R with or without associated native-pattern glycosylation. IL-15R expressed in yeast or mammalian expression systems, e.g., COS-7 cells, may be similar or slightly different in molecular weight and glycosylation pattern than the native molecules, depending upon the expression system. Expression of IL-15R DNAs in bacteria such as *E. coli* provides non-glycosylated molecules. Functional mutant analogs of mammalian IL-15R having inactivated N-glycosylation sites can be produced by  
10 oligonucleotide synthesis and ligation or by site-specific mutagenesis techniques. These analog proteins can be produced in a homogeneous, reduced-carbohydrate form in good yield using yeast expression systems. N-glycosylation sites in eukaryotic proteins are characterized by the amino acid triplet Asn-A<sub>1</sub>-Z, where A<sub>1</sub> is any amino acid except Pro, and Z is Ser or Thr. In this sequence, asparagine provides a side chain amino group for  
15 covalent attachment of carbohydrate. Such sites can be eliminated by substituting another amino acid for Asn or for residue Z, deleting Asn or Z, or inserting a non-Z amino acid between A<sub>1</sub> and Z, or an amino acid other than Asn between Asn and A<sub>1</sub>.

Subunits of IL-15R may be constructed by deleting terminal or internal residues or sequences. Particularly preferred sequences include those in which the transmembrane  
20 region and intracellular domain of IL-15R are deleted or substituted with hydrophilic residues to facilitate secretion of the receptor into the cell culture medium. Soluble IL-15R proteins may also include part of the transmembrane region, provided that the soluble IL-15R protein is capable of being secreted from the cell. The resulting protein is referred to as a soluble IL-15R molecule that retains its ability to bind IL-15. The present invention  
25 contemplates such soluble IL-15R constructs corresponding to all or part of the extracellular region of IL-15R. The resulting soluble IL-15R constructs are then inserted and expressed in appropriate expression vectors and assayed for the ability to bind IL-15, as described in Example 1. Biologically active soluble IL-15Rs (i.e., those which bind IL-15) resulting from such constructions are also contemplated to be within the scope of the  
30 present invention. Soluble IL-15Rs can be used to inhibit IL-15, for example, in ameliorating undesired effects of IL-15, *in vitro* or *in vivo*. For example, significant levels of IL-15 mRNA occur in kidney, lung, liver, and heart, organs that may be transplanted. Soluble IL-15Rs are thus likely to be useful as IL-15 antagonists in preventing or treating graft rejection. Soluble IL-15Rs can also be used as components of quantitative or  
35 qualitative assays for IL-15, or for affinity purification of IL-15.

Mutations in nucleotide sequences constructed for expression of the above-described variant or analog IL-15R proteins should, of course, preserve the reading frame phase of the coding sequences and preferably will not create complementary regions that

could hybridize to produce secondary mRNA structures such as loops or hairpins that would adversely affect translation of the receptor mRNA. Although a mutation site may be predetermined, it is not necessary that the nature of the mutation *per se* be predetermined. For example, in order to select for optimum characteristics of mutants at a given site, random mutagenesis may be conducted at the target codon and the expressed IL-15R mutants screened for the desired activity.

Not all mutations in the nucleotide sequence that encodes IL-15R will be expressed in the final product. For example, nucleotide substitutions may be made to enhance expression, primarily to avoid secondary structure loops in the transcribed mRNA (see EPA 75,444A, incorporated herein by reference), or to provide codons that are more readily translated by the selected host, e.g., the well-known *E. coli* preference codons for *E. coli* expression (see U.S. Patent 4,425,437, column 6). The known degeneracy of the genetic code permits variation of a DNA sequence without altering the amino acid sequence, since a given amino acid may be encoded by more than one codon.

Mutations can be introduced at particular loci by synthesizing oligonucleotides containing a mutant sequence, flanked by restriction sites enabling ligation to fragments of the native sequence. Following ligation, the resulting reconstructed sequence encodes an analog having the desired amino acid insertion, substitution, or deletion.

Alternatively, oligonucleotide-directed site-specific mutagenesis procedures can be employed to provide an altered gene having particular codons altered according to the substitution, deletion, or insertion required. Examples of methods of making the alterations set forth above are disclosed by Walder et al., *Gene* 42:133 (1986); Bauer et al., *Gene* 37:73 (1985); Craik, *BioTechniques*, 12-19 (1985); Smith et al., *Genetic Engineering: Principles and Methods*, Plenum Press (1981); and U.S. Patent Nos. 4,518,584 and 4,737,462.

The IL-15R proteins of the present invention encompass proteins encoded by (a) a DNA sequence derived from the coding region of a native IL-15R gene or (b) a DNA sequence capable of hybridization to a native IL-15R DNA of (a) under moderate to high stringency conditions and that encodes biologically active IL-15R. IL-15R proteins encoded by a DNA molecule that varies from the DNA sequences of SEQ ID NO:1, wherein one strand of the DNA molecule will hybridize to the DNA sequence presented in SEQ ID NO:1, include, but are not limited to, IL-15R fragments (soluble or membrane-bound) and IL-15R proteins comprising inactivated N-glycosylation site(s), inactivated KEX2 protease processing site(s), and/or conservative amino acid substitution(s), as described above. IL-15R proteins encoded by DNA derived from other mammalian species, wherein the DNA will hybridize to the murine DNA of SEQ ID NO:1, are also encompassed.

Both monovalent forms and polyvalent forms of IL-15R are useful in the compositions and methods of this invention. Polyvalent forms possess multiple IL-15R binding sites for IL-15 ligand. For example, a bivalent soluble IL-15R may consist of two tandem repeats of the extracellular region of IL-15R, separated by a linker region. Two IL-15R polypeptides (each capable of binding IL-15) may be joined by any suitable means, e.g., using one of the commercially available cross-linking reagents used to attach one polypeptide to another (Pierce Chemical Co., Rockford, IL). Alternatively, a fusion protein comprising multiple IL-15R polypeptides joined by peptide linkers may be produced using recombinant DNA technology. Suitable peptide linkers comprise a chain of amino acids, preferably from 20 to 100 amino acids in length. The linker advantageously comprises amino acids selected from the group consisting of glycine, asparagine, serine, threonine, and alanine. Examples of suitable peptide linkers and the use of such peptide linkers are found in U.S. Patent 5,073,627.

Alternate polyvalent forms may also be constructed, for example, by chemically coupling IL-15R to any clinically acceptable carrier molecule, a polymer selected from the group consisting of Ficoll, polyethylene glycol or dextran using conventional coupling techniques. Alternatively, IL-15R may be chemically coupled to biotin, and the biotin-IL-15R conjugate then allowed to bind to avidin, resulting in tetravalent avidin/biotin/IL-15R molecules. IL-15R may also be covalently coupled to dinitrophenol (DNP) or trinitrophenol (TNP) and the resulting conjugate precipitated with anti-DNP or anti-TNP-IgM, to form decameric conjugates with a valency of 10 for IL-15R binding sites.

A recombinant chimeric antibody molecule may also be produced having IL-15R sequences substituted for the variable domains of either or both of the immunoglobulin molecule heavy and light chains and having unmodified constant region domains. For example, chimeric IL-15R/IgG<sub>1</sub> may be produced from two chimeric genes -- an IL-15R/human  $\kappa$  light chain chimera (IL-15R/C $\kappa$ ) and an IL-15R/human  $\gamma$ 1 heavy chain chimera (IL-15R/C $\gamma$ -1). Following transcription and translation of the two chimeric genes, the gene products assemble into a single chimeric antibody molecule having IL-15R displayed bivalently. Such polyvalent forms of IL-15R may have enhanced binding affinity for IL-15 ligand. Additional details relating to the construction of such chimeric antibody molecules are disclosed in WO 89/09622 and EP 315062.

#### Expression of Recombinant IL-15R

The present invention provides recombinant expression vectors to amplify or express DNA encoding IL-15R. Recombinant expression vectors are replicable DNA constructs that have synthetic or cDNA-derived DNA fragments encoding mammalian IL-15R or bioequivalent analogs operably linked to suitable transcriptional or translational regulatory elements derived from mammalian, microbial, viral or insect genes. A

transcriptional unit generally comprises an assembly of (1) a genetic element or elements having a regulatory role in gene expression, for example, transcriptional promoters or enhancers, (2) a structural or coding sequence that is transcribed into mRNA and translated into protein, and (3) appropriate transcription and translation initiation and termination sequences, as described in detail below. Such regulatory elements may include an operator sequence to control transcription, a sequence encoding suitable mRNA ribosomal binding sites. The ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants may additionally be incorporated. DNA regions are operably linked when they are functionally related to each other. For example, DNA for a signal peptide (secretory leader) is operably linked to DNA for a polypeptide if it is expressed as a precursor that participates in the secretion of the polypeptide; a promoter is operably linked to a coding sequence if it controls the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to permit translation. Generally, operably linked means contiguous and, in the case of secretory leaders, contiguous and in reading frame. Structural elements intended for use in yeast expression systems preferably include a leader sequence enabling extracellular secretion of translated protein by a host cell. Alternatively, where recombinant protein is expressed without a leader or transport sequence, it may include an N-terminal methionine residue. This residue may optionally be subsequently cleaved from the expressed recombinant protein to provide a final product.

DNA sequences encoding mammalian IL-15Rs that are to be expressed in a microorganism will preferably contain no introns that could prematurely terminate transcription of DNA into mRNA. However, premature termination of transcription may be desirable, for example, where it would result in mutants having advantageous C-terminal truncations, for example, deletion of a transmembrane region to yield a soluble receptor not bound to the cell membrane. Due to code degeneracy, there can be considerable variation in nucleotide sequences encoding the same amino acid sequence. Other embodiments include sequences capable of hybridizing to SEQ ID NO:1 under at least moderately stringent conditions (50°C, 2x SSC) and other sequences hybridizing or degenerate to those that encode biologically active IL-15R polypeptides.

Recombinant IL-15R DNA is expressed or amplified in a recombinant expression system comprising a substantially homogeneous monoculture of suitable host microorganisms, for example, bacteria such as *E. coli* or yeast such as *S. cerevisiae*, that have stably integrated (by transformation or transfection) a recombinant transcriptional unit into chromosomal DNA or carry the recombinant transcriptional unit as a component of a resident plasmid. Mammalian host cells are preferred for expressing recombinant IL-15R. Generally, cells constituting the system are the progeny of a single ancestral transformant. Recombinant expression systems as defined herein will express heterologous protein upon

induction of the regulatory elements linked to the DNA sequence or synthetic gene to be expressed.

Transformed host cells are cells that have been transformed or transfected with IL-15R vectors constructed using recombinant DNA techniques. Transformed host cells ordinarily express IL-15R, but host cells transformed for purposes of cloning or amplifying IL-15R DNA do not need to express IL-15R. Expressed IL-15R will be deposited in the cell membrane or secreted into the culture supernatant, depending on the IL-15R DNA selected. Suitable host cells for expression of mammalian IL-15R include prokaryotes, yeast or higher eukaryotic cells under the control of appropriate promoters. Prokaryotes include gram negative or gram positive organisms, for example *E. coli* or bacilli. Higher eukaryotic cells include established cell lines of mammalian origin as described below. Cell-free translation systems could also be employed to produce mammalian IL-15R using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are described by Pouwels et al., *Cloning Vectors: A Laboratory Manual*, Elsevier, New York (1985).

Prokaryotic expression hosts may be used for expression of IL-15R that do not require extensive proteolytic and disulfide processing. Prokaryotic expression vectors generally comprise one or more phenotypic selectable markers, for example a gene encoding proteins conferring antibiotic resistance or supplying an autotrophic requirement, and an origin of replication recognized by the host to ensure amplification within the host. Suitable prokaryotic hosts for transformation include *E. coli*, *Bacillus subtilis*, *Salmonella typhimurium*, and various species within the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*, although others may also be employed as a matter of choice.

Useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well-known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 and pGEX (Pharmacia Fine Chemicals, Uppsala, Sweden) and pGEM1 (Promega Biotec, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed. *E. coli* is typically transformed using derivatives of pBR322, a plasmid derived from an *E. coli* species (Bolivar et al., *Gene* 2:95 (1977)). pBR322 contains genes for ampicillin and tetracycline resistance and thus provides simple means for identifying transformed cells.

Promoters commonly used in recombinant microbial expression vectors include the  $\beta$ -lactamase (penicillinase) and lactose promoter system (Chang et al., *Nature* 275:615 (1978); and Goeddel et al., *Nature* 281:544 (1979)), the tryptophan (trp) promoter system (Goeddel et al., *Nucl. Acids Res.* 8:4057 (1980); and EPA 36,776) and tac promoter



(Maniatis, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, p. 412 (1982)). A particularly useful bacterial expression system employs the phage  $\lambda$  P<sub>L</sub> promoter and cI857ts thermolabile repressor. Plasmid vectors available from the American Type Culture Collection that incorporate derivatives of the  $\lambda$  P<sub>L</sub> promoter include plasmid  
5 pHUB2, resident in *E. coli* strain JMB9 (ATCC 37092) and pPLc28, resident in *E. coli* RR1 (ATCC 53082).

Recombinant IL-15R proteins may also be expressed in yeast hosts, preferably from the *Saccharomyces* species, such as *S. cerevisiae*. Yeast of other genera, such as  
10 *Pichia* or *Kluyveromyces* may also be employed. Yeast vectors will generally contain an origin of replication from the 2 $\mu$  yeast plasmid or an autonomously replicating sequence (ARS), promoter, DNA encoding IL-15R, sequences for polyadenylation and transcription termination and a selection gene. Preferably, yeast vectors will include an origin of replication and selectable marker permitting transformation of both yeast and *E. coli*, e.g., the ampicillin resistance gene of *E. coli* and *S. cerevisiae* TRP1 or URA3 gene, that  
15 provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, and a promoter derived from a highly expressed yeast gene to induce transcription of a structural sequence downstream. The presence of the TRP1 or URA3 lesion in the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan or uracil.

20 Suitable promoter sequences in yeast vectors include the promoters for metallothionein, 3-phosphoglycerate kinase (Hitzeman et al., *J. Biol. Chem.* 255:2073 (1980)) or other glycolytic enzymes (Hess et al., *J. Adv. Enzyme Reg.* 7:149 (1968); and Holland et al., *Biochem.* 17:4900 (1978)). Suitable vectors and promoters for use in yeast expression are further described in R. Hitzeman et al., EPA 73,657.

25 Preferred yeast vectors can be assembled using DNA sequences from pUC18 for selection and replication in *E. coli* (Amp<sup>r</sup> gene and origin of replication) and yeast DNA sequences including a glucose-repressible ADH2 promoter and  $\alpha$ -factor secretion leader. The ADH2 promoter has been described by Russell et al., *J. Biol. Chem.* 258:2674 (1982) and Beier et al., *Nature* 300:724 (1982). The yeast  $\alpha$ -factor leader, that directs secretion  
30 of heterologous proteins, can be inserted between the promoter and the structural gene to be expressed (see, e.g., Kurjan et al., *Cell* 30:933 (1982); and Bitter et al., *Proc. Natl. Acad. Sci. USA* 81:5330 (1984)). The leader sequence may be modified to contain, near its 3' end, one or more useful restriction sites to facilitate fusion of the leader sequence to foreign genes. Suitable yeast transformation protocols are known to those of skill in the art  
35 (see Hinnen et al., *Proc. Natl. Acad. Sci. USA* 75:1929 (1978); Sherman et al., *Laboratory Course Manual for Methods in Yeast Genetics*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1986)).

Host strains transformed by vectors comprising the ADH2 promoter may be grown for expression in a rich medium consisting of 1% yeast extract, 2% peptone, and 1% or 4% glucose supplemented with 80 µg/ml adenine and 80 µg/ml uracil. Derepression of the ADH2 promoter occurs upon exhaustion of medium glucose. Crude yeast supernatants are harvested by filtration and held at 4°C prior to further purification.

Various mammalian or insect cell culture systems are also advantageously employed to express recombinant protein. Expression of recombinant proteins in mammalian cells is particularly preferred because such proteins are generally correctly folded, appropriately modified and completely functional. Examples of suitable mammalian host cell lines include the COS-7 lines of monkey kidney cells, described by Gluzman, *Cell* 23:175 (1981), and other cell lines capable of expressing a heterologous gene in an appropriate vector including, for example, L cells, C127, 3T3, Chinese hamster ovary (CHO), HeLa and BHK cell lines. Mammalian expression vectors may comprise nontranscribed elements such as an origin of replication, a suitable promoter and enhancer linked to the gene to be expressed, and other 5' or 3' flanking nontranscribed sequences, and 5' or 3' nontranslated sequences, such as necessary ribosome binding sites, a polyadenylation site, splice donor and acceptor sites, and transcriptional termination sequences. Baculovirus systems for production of heterologous proteins in insect cells are reviewed by Luckow and Summers, *Bio/Technology* 6:47 (1988).

The transcriptional and translational control sequences in expression vectors to be used in transforming vertebrate cells may be provided by viral sources. For example, commonly used promoters and enhancers are derived from Polyoma, Adenovirus 2, Simian Virus 40 (SV40), and human cytomegalovirus. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early and late promoter, enhancer, splice, and polyadenylation sites may be used to provide the other genetic elements required for expression of a heterologous DNA sequence. The early and late promoters are particularly useful because both are obtained easily from the virus as a fragment that also contains the SV40 viral origin of replication (Fiers et al., *Nature* 273:113 (1978)). Smaller or larger SV40 fragments may also be used, provided the approximately 250 bp sequence extending from the *Hind* III site toward the *Bgl* I site located in the viral origin of replication is included. Further, mammalian genomic IL-15R promoter, control and/or signal sequences may be used, provided such control sequences are compatible with the host cell chosen. Exemplary vectors can be constructed as disclosed by Okayama and Berg, *Mol. Cell. Biol.* 3:280 (1983).

A useful system for stable high level expression of mammalian receptor cDNAs in C127 murine mammary epithelial cells can be constructed substantially as described by Cosman et al., *Mol. Immunol.* 23:935 (1986).

In preferred aspects of the present invention, recombinant expression vectors comprising IL-15R cDNAs are stably integrated into a host cell's DNA. Elevated levels of expression product are achieved by selecting for cell lines having amplified numbers of vector DNA. Cell lines having amplified numbers of vector DNA are selected, for example, by transforming a host cell with a vector comprising a DNA sequence that encodes an enzyme that is inhibited by a known drug. The vector may also comprise a DNA sequence that encodes a desired protein. Alternatively, the host cell may be co-transformed with a second vector that comprises the DNA sequence that encodes the desired protein. The transformed or co-transformed host cells are then cultured in increasing concentrations of the known drug, thereby selecting for drug-resistant cells. Such drug-resistant cells survive in increased concentrations of the toxic drug by overproduction of the enzyme that is inhibited by the drug, frequently as a result of amplification of the gene encoding the enzyme. Where drug resistance is caused by an increase in the copy number of the vector DNA encoding the inhibiting enzyme, there is a concomitant co-amplification of the vector DNA encoding the desired protein (e.g., IL-15R) in the host cell's DNA.

A preferred system for such co-amplification uses the gene for dihydrofolate reductase (DHFR), that can be inhibited by the drug methotrexate (MTX). To achieve co-amplification, a host cell that lacks an active gene encoding DHFR is either transformed with a vector that comprises DNA sequence encoding DHFR and a desired protein, or is co-transformed with a vector comprising a DNA sequence encoding DHFR and a vector comprising a DNA sequence encoding the desired protein. The transformed or co-transformed host cells are cultured in media containing increasing levels of MTX, and those cell lines that survive are selected.

A particularly preferred co-amplification system uses the gene for glutamine synthetase (GS), that is responsible for the synthesis of glutamine from glutamate and ammonia using the hydrolysis of ATP to ADP and phosphate to drive the reaction. GS is subject to inhibition by a variety of inhibitors, for example methionine sulfoximine (MSX). Thus, IL-15R can be expressed in high concentrations by co-amplifying cells transformed with a vector comprising the DNA sequence for GS and a desired protein, or co-transformed with a vector comprising a DNA sequence encoding GS and a vector comprising a DNA sequence encoding the desired protein, culturing the host cells in media containing increasing levels of MSX and selecting for surviving cells. The GS co-amplification system, appropriate recombinant expression vectors and cells lines, are described in the following PCT applications: WO 87/04462, WO 89/01036, WO 89/10404 and WO 86/05807.

Recombinant proteins are preferably expressed by co-amplification of DHFR or GS in a mammalian host cell, such as Chinese Hamster Ovary (CHO) cells, or alternatively in a

murine myeloma cell line, such as SP2/0-Ag14 or NS0 or a rat myeloma cell line, such as YB2/3.0-Ag20, disclosed in PCT applications WO 89/10404 and WO 86/05807.

Vectors derived from retroviruses may be employed in mammalian host cells. A preferred retroviral expression vector is tgLS(+) HyTK, described in PCT application  
5 WO 92/08796.

A preferred eukaryotic vector for expression of IL-15R DNA is disclosed below in Example 1. This vector, referred to as pDC304, was derived from pDC302 previously described by Mosley et al., *Cell*, 59: 335-348 (1989) by deleting the adenovirus tripartite leader in pDC302.

10

#### Sense and Antisense Sequences

The present invention provides both double-stranded and single-stranded IL-15R DNA, and IL-15R mRNA as well. The single-stranded IL-15R nucleic acids have use as probes to detect the presence of hybridizing IL-15R nucleic acids (e.g., in *in vitro* assays) and as sense and antisense molecules to block expression of IL-15R.  
15

In one embodiment, the present invention provides antisense or sense molecules comprising a single-stranded nucleic acid sequence (either RNA or DNA) capable of binding to target IL-15R mRNA (sense) or IL-15R DNA (antisense) sequences. These antisense or sense molecules may comprise a fragment of the coding region of IL-15R cDNA, and, in one embodiment, are oligonucleotides comprising at least about 14 nucleotides, preferably from about 14 to about 30 nucleotides, of an IL-15R cDNA sequence. The ability to create an antisense or sense oligonucleotide based upon a cDNA sequence for a given protein is described in, for example, Stein and Cohen, *Cancer Res.* 48:2659 (1988) and van der Krol et al., *BioTechniques* 6:958 (1988).  
20

Binding of antisense or sense oligonucleotides to target nucleic acid sequences results in the formation of duplexes that block translation (RNA) or transcription (DNA) by one of several means, including enhanced degradation of the duplexes, premature termination of transcription or translation, or by other means. The oligonucleotides thus may be used to block expression of IL-15R proteins. Uses of the antisense and sense nucleic acid sequences include, but are not limited to, use as research reagents. The biological effects of blocking  
25 30

IL-15R expression in cultured cells may be studied, for example. The oligonucleotides also may be employed in developing therapeutic procedures that involve blocking IL-15R expression *in vivo*.

35 Antisense or sense oligonucleotides further comprise oligonucleotides having modified sugar-phosphodiester backbones (or other sugar linkages, such as those described in WO 91/06629) and wherein such sugar linkages are resistant to endogenous nucleases. Such oligonucleotides with resistant sugar linkages are relatively stable *in vivo*

(i.e., capable of resisting enzymatic degradation) but retain sequence specificity for binding to target nucleotide sequences. Other examples of sense or antisense oligonucleotides include those oligonucleotides that are covalently linked to organic moieties such as those described in

5 WO 90/10448, or to other moieties that increase affinity of the oligonucleotide for a target nucleic acid sequence, such as poly-(L-lysine). Further still, intercalating agents, such as ellipticine, and alkylating agents or metal complexes may be attached to sense or antisense oligonucleotides to modify binding specificities of the antisense or sense oligonucleotide for the target nucleotide sequence.

10 Antisense or sense oligonucleotides may be introduced into a cell containing the target nucleic acid sequence by any suitable method, including, for example,  $\text{CaPO}_4$ -mediated DNA transfection, electroporation, or by using gene transfer vectors such as Epstein-Barr virus. A preferred method involves insertion of the antisense or sense oligonucleotide into a suitable retroviral vector, then contacting the target cell with the  
15 retrovirus vector containing the inserted sequence, either *in vivo* or *ex vivo*. Suitable retroviral vectors include, but are not limited to, the murine retrovirus M-MuLV, N2 (a retrovirus derived from M-MuLV), or the double copy vectors designated DCT5A, DCT5B and DCT5C (see PCT Application US 90/02656).

20 Sense or antisense oligonucleotides also may be introduced into a cell containing the target nucleotide sequence by attaching the oligonucleotide to a molecule that binds to the target cell, as described in WO 91/04753. The oligonucleotide may be attached to molecules that include, but are not limited to, antibodies, growth factors, other cytokines, or other ligands that bind to cell surface receptors.

25 Alternatively, a sense or an antisense oligonucleotide may be introduced into a cell containing the target nucleic acid sequence by formation of an oligonucleotide-lipid complex, as described in WO 90/10448. The sense or antisense oligonucleotide-lipid complex is preferably dissociated within the cell by an endogenous lipase.

30 The following examples are offered by way of illustration, and not by way of limitation. Those skilled in the art will recognize that variations of the invention embodied in the examples can be made, especially in light of the teachings of the various references cited herein, the disclosures of which are incorporated by reference.

## 35 EXAMPLES

### Example 1

#### Isolation and Expression of cDNAs Encoding Murine IL-15R

40 A. Radiolabeling of IL-15. Recombinant flag simian IL-15 expressed in yeast (SEQ ID NO:3) was purified by passage over a Phenyl Sepharose CL-4B column

(Pharmacia, Piscataway, NJ) followed by two passages over reverse phase HPLC C4 columns (Vydac), the first using a pyridine acetate/propanol buffer system, the second in trifluoro-acetic acid acetonitrile system. Fractions containing pure IL-15 were dried under nitrogen and radiolabeled using the enzymobead iodination reagent (BioRad, Richmond VA) as described by in Park et al., *J. Exp. Med.*, 165:1201-1206 (1987). The biological activity of radiolabeled IL-15 was assessed using the mitochondrial stain MTT (3-4, 5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; thiazol blue (Sigma, St. Dextran, as described by Cosman et al., *Nature*, 312:768-771 (1984)).

B. Binding To Intact Cells. A source for IL-15R was selected by screening various murine and human cells lines and tissues for expression of IL-15R based on their ability to bind  $^{125}\text{I}$ -IL-15 that was prepared as described above in Example 1A. For the binding assays, a phthalate oil separation method (Dower et al., *J. Immunol.* 132:751 (1984)) was performed as described by Park et al., *J. Biol. Chem* 261:4177 (1986) and Park et al., *Proc. Natl. Acad. Sci. USA* 84:5267 (1987) on candidate cells grown in suspension culture. Nonspecific binding of  $^{125}\text{I}$ -IL-15 was measured in the presence of a 200-fold or greater molar excess of unlabeled IL-15. Sodium azide (0.2%) was included in all binding assays to inhibit internalization of  $^{125}\text{I}$ -IL-15 at 37°C. Activated PBT and well as antigen specific T cell clones expressed only a few hundred receptors for IL-15. Cells from the murine Th2 CD4<sup>+</sup> cell clone, D10 (Kaye et al., *J. Immunol.*, 133:1339 (1984)), expressed up to 24,000 IL-15 receptors when cultured with IL-2.

C. Construction and Screening of cDNA Library. Polyadenylated mRNA was prepared from a D10 cell line and cDNAs were prepared using standard techniques. The D10 line is a producer of murine IL-15R. cDNA ends were adapted with *Bgl* II adaptors:  
5' -GATCTTGGAAACGAGACGACCTGCT-3' (SEQ ID NO:4)  
3' -AACCTTGCTCTGCTGGACGA-5' (SEQ ID NO:5)  
and cloned into vector pDC304.

COS-7 cells were transfected with miniprep DNA from pools of cDNA clones directly on glass slides and cultured for 2-3 days to permit transient expression of IL-15R. The slides containing the transfected cells were then incubated with medium containing  $^{125}\text{I}$ -labeled IL-15, washed to remove unbound labeled IL-15, fixed with glutaraldehyde, and dipped in liquid autoradiographic emulsion and exposed in the dark. After developing the slides, they were individually examined with a microscope and positive cells expressing IL-15R were identified by the presence of autoradiographic silver grains against a light background. Approximately 20,000 cDNAs were screened in pools of approximately 1000 cDNAs each using the slide autoradiographic method until assay of one transfectant pool showed multiple cells clearly positive for IL-15 binding. This pool was then partitioned into pools of approximately 100 and again screened by slide autoradiography and a positive pool was identified. Individual colonies from this pool of approximately 100

were screened until a single clone (clone D1-4-D5) was identified that directed synthesis of a surface protein with detectable IL-15 binding activity. This clone was isolated and sequenced to determine the sequence of the murine IL-15R cDNA clone, D1-4-D5. The cloning vector pDC304 containing the murine IL-15R cDNA clone, D1-4-D5, was deposited with the American Type Culture Collection ("ATCC") under accession number ATCC 62659. The murine IL-15R cDNA insert is made up of a 71-bp 5' noncoding region before an open reading frame of 792 bp and a 995-bp 3' non coding region. The nucleotide sequence of the open reading frame is disclosed in SEQ ID NO:1. The amino acid sequence of a full length murine IL-15R (i.e., including signal peptide, extracellular domain, transmembrane region and cytoplasmic domain) is shown in SEQ ID NOs:1 and 2. The amino acid sequence in SEQ ID NOs:1 and 2 predicts a type 1 membrane protein (i.e., a single transmembrane region with a N-terminal extracellular domain and a C-terminal cytoplasmic domain). A predicted signal peptide cleavage occurs between amino acids 30 and 31 in SEQ ID NO:2; amino acids 32 and 33 are predicted to form another, preferred, cleavage site. The predicted transmembrane region includes amino acids 206 to 226 in SEQ ID NO:2.

D. Recombinant IL-15R Binding. Plasmid DNA from IL-15 receptor expression plasmid was used to transfect a sub-confluent layer of monkey COS-7 cells using DEAE-dextran followed by chloroquine treatment, as described by Luthman et al., *Nucl Acids Res.* 11:1295 (1983) and McCutchan et al., *J. Natl. Cancer Inst.* 41:351 (1968). The cells were then grown in culture for three days to permit transient expression of the inserted sequences. After three days, the cell monolayers were assayed for <sup>125</sup>I-IL-15 binding essentially as described by Park, et al., *J. Exp. Med.* 166:476 (1987). Non-specific binding of <sup>125</sup>I-IL-15 was measured in the presence of 200-fold or greater excess of unlabeled IL-15. Initial binding studies of <sup>125</sup>I-IL-15 to COS cells transfected with IL-15R cDNA clone D1-4-D5 showed very high levels of expression (~500,000 sites/cell), with an estimated affinity of 1.0 - 2.2 nM, which is much lower than the affinity of the native receptor on D10 cells.

E. Soluble IL-15R. A soluble murine IL-15 receptor was prepared by deleting the transmembrane and cytoplasmic domains, with the C-terminal end corresponding to Thr at amino acid 204 of SEQ ID NO:1, and adding 5 C-terminal Histidines. The soluble IL-15 receptor was biologically active, as demonstrated by the fact that it inhibited binding of radiolabeled IL-15 to cells expressing membrane bound IL-15 receptor (Figure 1).

## Example 2

### Preparation of Monoclonal Antibodies to IL-15R

Preparations of purified recombinant IL-15R, for example, human IL-15R, or transfected COS cells expressing high levels of IL-15R are employed to generate

monoclonal antibodies against IL-15R using conventional techniques, for example, those disclosed in U.S. Patent 4,411,993. Such antibodies are likely to be useful in interfering with IL-15 binding to IL-15R, for example, in ameliorating undesired effects of IL-15, or as components of diagnostic or research assays for IL-15 or soluble IL-15R.

- 5 To immunize mice or rats, IL-15R immunogen is emulsified in complete Freund's adjuvant and injected in amounts ranging from 10-100 µg, subcutaneously. Ten to twenty-one days later, the immunized animals are boosted with additional immunogen emulsified in incomplete Freund's adjuvant and periodically boosted thereafter on a weekly to biweekly immunization schedule. Serum samples are periodically taken by retro-orbital  
10 bleeding or tail-tip excision for testing by dot-blot assay (antibody sandwich) or ELISA (enzyme-linked immunosorbent assay). Other assay procedures are also suitable. Following detection of an appropriate antibody titer, positive animals are given an intravenous injection of antigen in saline. Three to four days later, the animals are sacrificed, splenocytes harvested, and fused with an appropriate murine myeloma cell line.  
15 Hybridoma cell lines generated by this procedure are plated in multiple microtiter plates in a HAT selective medium (hypoxanthine, aminopterin, and thymidine) to inhibit proliferation of non-fused cells, myeloma hybrids, and spleen cell hybrids.

- Hybridoma clones thus generated can be screened by ELISA for reactivity with IL-15R, for example, by adaptations of the techniques disclosed by Engvall et al.,  
20 *Immunochem.* 8:871 (1971) and in U.S. Patent 4,703,004. Clones that produce antibodies that bind IL-15R and inhibit binding of IL-15 to IL-15R (blocking or neutralizing antibodies) can also be isolated. Positive clones are then injected into the peritoneal cavities of syngeneic animals to produce ascites containing high concentrations (>1 mg/ml) of anti-IL-15R monoclonal antibody. The resulting monoclonal antibody can  
25 be purified by ammonium sulfate precipitation followed by gel exclusion chromatography, and/or affinity chromatography based on binding of antibody to Protein A of *Staphylococcus aureus*.

### 30 Example 3

#### Isolation and Expression of cDNAs Encoding Human IL-15R

##### A. Binding of IL-15 to human cells

- Various human cell lines and tissues were screened for the ability to bind  
35 radiolabeled IL-15 substantially as described in Example 1. High affinity binding was observed on activated peripheral blood mononuclear cells, activated monocytes and some EBV-transformed cell lines. High affinity binding was also measured on human fibroblast lines such as WI26-VA4 (ATCC CCL 95.1; Kd: 27 pM; 1,400 sites per cell), a glioblastoma cell line, A-172 (ATCC CRL 1620; Kd 50-138 pM; 1,560-4,350 sites pre  
40 cell), and vascular endothelial cells (both venous and arterial origin, Kd 33 pM, 990 sites



per cell; Kd 163 pM, 1,920 sites per cell, respectively). Cross-linking of radiolabeled IL-15 to receptors present on the surface of A172 cells showed a major IL-15 binding protein with an estimated molecular weight of 55-65 Kd, a size similar to that seen on the D10 murine cell line by cross-linking.

5           B.     Clone W5

A cDNA encoding human IL-15R was isolated by cross-species hybridization of the murine IL-15R cDNA to a human cDNA library prepared from the human cell line WI26-VA4-VA4 in the bacteriophage  $\lambda$ gt10 vector. Preparation of the library is described in U.S. patent 5,194,375, issued March 16, 1993 (DNAs Encoding IL-7 Receptors). The  
10   WI26-VA4 library was screened with a random prime labeled murine IL-15R cDNA probe in 50% formamide buffer (50% formamide, 5x SSC, 20 mM EDTA, 2x Denhardt's, 1% SDS, 0.1% sarcosyl, 50 mM KHPO<sub>4</sub> pH 6.5, 300  $\mu$ g/ml salmon sperm DNA) using 1 x 10<sup>6</sup> cpm of probe/ml of hybridization solution, at 42°C for 16-20 hours. The filters were washed once with 6x SSC/0.1% SDS at room temperature, followed by several moderate  
15   stringency washes in 2x SSC/0.1% SDS at 55°C.

Approximately 500,000 plaques of the amplified  $\lambda$ gt10/WI26-VA4 library were screened by standard methods, using the random-prime labeled murine IL-15R probe, which contained the entire coding region as well as 5' and 3' flanking non-coding regions. A single hybridizing plaque was identified, plaque-purified, and its cDNA insert amplified  
20   by PCR, purified, and sequenced. This clone, designated 'W5,' shared about 65% identity at the nucleotide level and 56% identity at the amino acid level with the murine cDNA, 'D1-4-D5' (SEQ ID NO: 1). The nucleotide and predicted amino acid sequence of W5 are shown in SEQ ID NOs:6 and 7.

As compared to the full-length murine clone D1-4-D5, W5 appeared to be missing a  
25   small portion of the expected 5' sequences, i.e., about 125 bp compared to the murine clone, indicating that W5 did not contain the coding region for the first part of a putative IL-15R signal peptide (missing 19 amino acids compared to the murine clone). The amino acid sequence in SEQ ID NOs:6 and 7 predicts a type 1 membrane protein (i.e., a single transmembrane region with an N-terminal extracellular domain and a C-terminal  
30   cytoplasmic domain). The predicted transmembrane region includes amino acids 190 to 210 of SEQ ID NOs:6 and 7. Binding of IL-15R to IL-15 is mediated through the extracellular domain of IL-15R as shown in Figure 2, all or portions of which are involved in binding.

A signal peptide cleavage is predicted to occur between amino acids 14 and 15 in  
35   SEQ ID NO:6. For murine IL-15 receptor, a signal peptide cleavage is predicted to occur between amino acids 32 and 33 in SEQ ID NO:2, or alternatively, between amino acids 30 and 31 in SEQ ID NO:2. Because of the similarity between murine and human IL-15 receptor in this region, and because the murine IL-7 leader sequence (see below) utilizes a

Thr residue as the mature N-terminal amino acid following the leader, the Thr at residue 12 of SEQ ID NOs:6 and 7 was chosen as the mature N-terminus of a human IL-15 receptor construct.

5 The mature peptide coding domain of W5 (nucleotides 34 through 753 of SEQ ID NO:6), and the remaining 3' non-coding sequence, was fused to the coding domain for the signal peptide of murine IL-7 in the expression vector pDC206 (similar to pDC201, described in Sims et al., *Science* 241: 585, 1988, with the addition of the murine IL-7 leader sequence, which is described in U.S. patent 4,965,195, issued October 23, 1990). Transfection of this recombinant plasmid into COS-7 cells followed by cell-surface binding  
10 of radiolabeled human IL-15 substantially as described in Example 1 showed that this plasmid encoded a biologically active polypeptide, i.e., one which bound IL-15. The clone W5 construct containing the murine IL-7 leader sequence in pDC206 was deposited with the American Type Culture Collection ("ATCC", 12301 Parklawn Dr., Rockville, MD 20852, USA), under the conditions of the Budapest Treaty on September 1, 1994, and  
15 given accession number ATCC 69690.

C. Clone P1

A  $\lambda$ gt10 library from human peripheral blood lymphocytes, prepared as described in USSN 08/094,669, filed July 20, 1993, and in Idzerda et al., *J. Exp. Med.* 171:861 (1990), was screened for the presence of a full-length clone encoding human IL-15R using  
20 a random prime labeled human IL15R cDNA probe consisting of the entire W5 cDNA without the poly-A tail (which had been removed by digestion of the cDNA with Ssp I followed by gel purification of the remaining fragment, resulting in a fragment of approximately 1465 bp), using substantially the same conditions as described for screening of the A172 library (described below). The resulting sequence of the cDNA insert from  
25 this clone (SEQ ID NOs:8 and 9) exhibited an in-frame insertion of 153 basepairs at the mature N-terminus (amino acids 24 through 74 of SEQ ID NOs:8 and 9), an in-frame deletion of 99 basepairs downstream of the mature N-terminus that deleted nucleotides 236 through 334 of SEQ ID NO:6 (the sequence encoding amino acids 79 through 112, with the substitution of a Lys residue encoded by the codon AAG, the equivalent of nucleotides 235,  
30 335 and 336 of SEQ ID NO:6), and also contained additional 5' sequence as compared to clone W5 (amino acids 1 through 10 of SEQ ID NOs:8 and 9), but still did not contain an initiator Met.

D. Clone A212

A library prepared from A172 cells as described in USSN 08/265,086, filed June  
35 17, 1994, was screened for the presence of a full-length clone encoding human IL-15R. DNA (1-5  $\mu$ g) from library pools (approximately 1000 cDNA clones/pool) was digested with Sal I to release the inserted DNA, electrophoresed (1% agarose, Tris-borate gel), and blotted to a nylon membrane. The blot was probed with a random prime labeled human

IL15R cDNA probe consisting of the entire W5 cDNA minus the poly A tail, under conditions of high stringency (50% formamide, 42°C hybridization for 16-20 hr, followed by washing at 2x SSC at room temperature for 5 minutes followed by 0.1x SSC/0.1% SDS at 68°C). The blot was autoradiographed, and a pool with a positive signal (i.e. hybridizing band) was chosen for isolation of individual clones by colony hybridization.

A portion of the frozen glycerol stock of the pool of cDNA clones corresponding to the Southern blot signal was diluted and plated onto appropriate plates (LB + ampicillin). Colonies were lifted onto nylon membranes and the membranes processed using standard techniques. The membranes were hybridized and washed under stringent conditions as described above, and a colony corresponding to a positive hybridizing signal was grown, its plasmid DNA purified and sequenced. The resulting sequence of the cDNA insert from this clone (SEQ ID NOs:10 and 11) exhibited the same in-frame deletion of 99 basepairs downstream of the mature N-terminus as clone P1 (a deletion of nucleotides 236 through 34 of SEQ ID NO:6, the sequence encoding amino acids 79 through 112, with the substitution of a Lys residue encoded by the codon AAG, the equivalent of nucleotides 235, 335 and 336 of SEQ ID NO:6). The plasmid was transfected into COS cells, and the ability of the encoded protein to bind IL-15 determined using slide autoradiography with <sup>125</sup>I-labeled human IL-15 substantially as described in Example 1. Clone A212 also encoded a biologically active polypeptide, i.e., one which bound IL-15. Additionally, clone A212 exhibited a complete signal peptide as compared to clone W5, as indicated by the presence of additional 5' sequence and an initiator Met.

#### E. Clone A133

A second clone was isolated from the A172 library described above, under substantially the same conditions. The nucleotide and amino acid sequence of the A133 clone are shown in SEQ ID NOs:12 and 13. This clone exhibited an incomplete 5' region which began at the equivalent of nucleotide 355 of clone W5 (SEQ ID NO:6), and an in-frame insertion downstream of the transmembrane region that results in a different cytoplasmic tail coding domain (amino acids 97 through 117 of SEQ ID NOs:12 and 13). A hybrid construct encoding the 5' half of W5 fused to A133 to give the alternate cytoplasmic tail (SEQ ID NO:14) was prepared, and expressed substantially as described above for clone W5. Cell-surface binding experiments using radiolabeled human IL-15 substantially as described in Example 1 showed that this hybrid construct encoded a polypeptide that bound IL-15.

SEQ ID NO:15 presents the predicted amino acid sequence of a composite human IL-15R containing the signal peptide of clone A212 and the coding region of clone W5. SEQ ID NO:15 also contains an Xaa at amino acid 182, wherein Xaa is Asn or Thr. Clones W5 and P1 contain a Thr at the equivalent position (W5: amino acid 166 of SEQ ID NOs:6 and 7; P1: amino acid 194 of SEQ ID NOs:8 and 9), whereas clones A212 and

A133 contain an Asn at the equivalent position (A212: amino acid 149 of SEQ ID NOs:10 and 11; A133: amino acid 48 of SEQ ID NOs:13 and 14). The Asn/Thr substitution does not affect binding of IL-15, as evidenced by the fact that both clones W5 and A212 encoded a peptide that bound IL-15, and may be due to allelic variation.

#### Example 4

##### Characterization of the role of the $\alpha$ subunit of the IL-15R

##### A. Functional role for the $\alpha$ subunit of the IL-15R on murine cells

In initial binding experiments with COS-7 cells transfected with the murine IL-15R $\alpha$  cDNA clone D5, in excess of  $5 \times 10^5$  receptors/cell were detected, a level too high to obtain accurate measurements of IL-15 binding to these cells. More accurate measurements of the affinity of IL-15R $\alpha$  for IL-15 were obtained using the murine IL-3 dependent 32D cell line, which constitutively expresses the IL-2R  $\alpha$  and  $\gamma_c$  chains, but failed to respond to IL-15 (Grabstein, et al., 1994, *supra*) as a model system. 32D cells stably expressing various components of the IL-2 and IL-15 receptors were derived and tested for their ability to proliferate in response to IL-15.

The original 32D cell line responded to IL-2, but a subline, 32D-01, which had lost the ability to respond to IL-2 (presumably because it no longer expressed sufficient levels of IL-2R $\beta$ ) was used in these experiments. The murine IL-2R $\beta$  chain was introduced into 32D-01, resulting in a line designated 32Dm $\beta$ -5, which had the ability to proliferate in response to IL-2 but not IL-15. No detectable IL-15 binding to 32D-01 or 32Dm $\beta$ -5 was seen by cytofluorometric analysis, suggesting that the level of IL-15R $\alpha$  was very low on these cells. Direct binding with  $^{125}$ I-IL-15 confirmed this result (see below).

To test the role of IL-15R $\alpha$ , 32D-01 cells were transfected with the IL-15R $\alpha$  cDNA, which resulted in a line expressing the  $\alpha$  chain, 32Dm15R $\alpha$ -102. Although these cells bound high levels of IL-15 as evidenced by both cytofluorometric analysis and radiolabeled IL-15 binding, they were unable to proliferate in response to IL-15. The 32Dm15R $\alpha$ -102 cells, like the parental 32D-01, did not express detectable levels of IL-2R $\beta$ . A cell line termed 32Dm $\beta$ m15R $\alpha$ -3, co-expressing both IL-15R $\alpha$  and IL-2R $\beta$  ( $\gamma_c$  is constitutively expressed) was derived, which was able to proliferate in response to IL-15 and IL-2, with a pattern similar to proliferation of the D10 cell line (from which D1-4-D5 ["D5"] was cloned). This result demonstrates that the ability of murine cells to respond to simian IL-15 is dependent on the level of IL-15R $\alpha$  expression and confirms the requirement for IL-2R $\beta$ .

##### B. IL-15R $\alpha$ binds IL-15 with high affinity

Preliminary equilibrium binding experiments with  $^{125}$ I-simian IL-15 indicated that the IL-15R $\alpha$  chain alone was binding IL-15 with very high affinity; therefore, the optimal

binding conditions necessary to accurately measure this affinity under equilibrium conditions, as well as to measure whether a receptor complex containing the  $\beta$  and  $\gamma_c$  chains along with the IL-15R $\alpha$  chain exhibited an enhanced affinity for IL-15, were assessed. The parental 32D-01 cell line expressed an average of  $100 \pm 33$  IL-15 binding sites per cell, with an affinity ( $K_a$ ) of  $1.4 \pm 0.4 \times 10^{11} \text{ M}^{-1}$ , which is similar to the affinity of IL-2 binding to the IL-2R $\alpha/\beta/\gamma_c$  complex. The 32Dm15R $\alpha$ -102 cells, transfected with the IL-15R $\alpha$  chain, exhibited a much higher level of IL-15 binding with the same very high affinity (average of  $15300 \pm 3700$  sites per cell with a  $K_a$  of  $1.5 \pm 0.9 \times 10^{11} \text{ M}^{-1}$ ). Given the low expression of IL-2R $\beta$  on these cells, the majority of these sites must reflect binding to the IL-15R $\alpha$  chain alone. This suggests that the low amount of IL-15 binding on the 32D-01 cells is due to endogenous IL-15R $\alpha$ .

The affinity of the receptors on both of these 32D lines is very similar to the affinity of the native IL-15R on the D10 cells from which the IL-15R $\alpha$  subunit was cloned (average  $K_a$  of  $1.3 \pm 0.5 \times 10^{11} \text{ M}^{-1}$ ). Although D10 cells express several hundred copies of IL-2R $\beta$ , inferred from the number of high affinity IL-2 binding sites ( $\sim 500$  sites/cell), a second component of binding in these cells which might correspond to a higher affinity  $\alpha/\beta$  or  $\alpha/\beta/\gamma_c$  complex was not detected. This result was substantiated by analysis of the 32Dm $\beta$ m15R $\alpha$ -3 cells, co-expressing both recombinant IL-15R $\alpha$  and IL-2R $\beta$  subunits. These cells showed binding characteristics very similar to those exhibited by the 32Dm15R $\alpha$ -102 cells, with an average  $K_a$  of  $2.2 \pm 0.3 \times 10^{11} \text{ M}^{-1}$ , and  $12800 \pm 2700$  receptors/cell.

In both D10 and 32Dm $\beta$ m15R $\alpha$ -3 cells, overexpression of the IL-15R $\alpha$  relative to the  $\beta$  subunit might serve to obscure a small higher affinity component. This possibility was addressed by analyzing binding to the 32Dm $\beta$ -5 cell line, which had been transfected with the  $\beta$  subunit alone. These cells showed a single high affinity binding site that was essentially identical to the parental 32D-01 line, with an average  $K_a$  of  $1.9 \pm 0.5 \times 10^{11} \text{ M}^{-1}$  and  $40 \pm 15$  sites per cell, presumably due to low level expression of endogenous IL-15R $\alpha$ . The observation that the 32Dm $\beta$ -5 cell line did not display any additional IL-15 binding sites relative to the 32D-01 parent line indicated that simian IL-15 is unable to bind with any detectable affinity to complexes of murine  $\beta$  and  $\gamma_c$ , in the absence of the IL-15R $\alpha$  chain.

## SEQUENCE LISTING

- 5 (1) GENERAL INFORMATION:
- (i) APPLICANT: Anderson, Dirk M  
Giri, Judith G
- 10 (ii) TITLE OF INVENTION: Interleukin-15 Receptors
- (iii) NUMBER OF SEQUENCES: 15
- (iv) CORRESPONDENCE ADDRESS:
- 15 (A) ADDRESSEE: Immunex Corporation  
(B) STREET: 51 University Street  
(C) CITY: Seattle  
(D) STATE: Washington  
(E) COUNTRY: USA  
20 (F) ZIP: 98101
- (v) COMPUTER READABLE FORM:
- (A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: Apple Macintosh  
25 (C) OPERATING SYSTEM: Apple Operating System 7.1  
(D) SOFTWARE: Microsoft Word for Apple, Version 5.1a
- (vi) CURRENT APPLICATION DATA:
- (A) APPLICATION NUMBER:  
30 (B) FILING DATE:  
(C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
- (A) APPLICATION NUMBER: USSN 08/300,903  
35 (B) FILING DATE: 06-SEPTEMBER-1994  
(C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
- (A) APPLICATION NUMBER: USSN 08/236,919  
40 (B) FILING DATE: 06-MAY-1994  
(C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
- (A) NAME: Perkins, Patricia Anne  
45 (B) REGISTRATION NUMBER: 34,693  
(C) REFERENCE/DOCKET NUMBER: 2822-WO
- (ix) TELECOMMUNICATION INFORMATION:
- (A) TELEPHONE: 206-587-0430  
50 (B) TELEFAX: 206-233-0644
- (2) INFORMATION FOR SEQ ID NO:1:
- (i) SEQUENCE CHARACTERISTICS:
- 55 (A) LENGTH: 792 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- 60 (ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

5

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..789

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

15	ATG GCC TCG CCG CAG CTC CGG GGC TAT GGA GTC CAG GCC ATT CCT GTG Met Ala Ser Pro Gln Leu Arg Gly Tyr Gly Val Gln Ala Ile Pro Val	48
	1 5 10 15	
20	TTG CTG CTG CTG CTG TTG CTA CTG TTG CTC CCG CTG AGG GTG ACG CCG Leu Leu Leu Leu Leu Leu Leu Leu Leu Leu Pro Leu Arg Val Thr Pro	96
	20 25 30	
25	GGC ACC ACG TGT CCA CCT CCC GTA TCT ATT GAG CAT GCT GAC ATC CCG Gly Thr Thr Cys Pro Pro Pro Val Ser Ile Glu His Ala Asp Ile Arg	144
	35 40 45	
30	GTC AAG AAT TAC AGT GTG AAC TCC AGG GAG AGG TAT GTC TGT AAC TCT Val Lys Asn Tyr Ser Val Asn Ser Arg Glu Arg Tyr Val Cys Asn Ser	192
	50 55 60	
35	GGC TTT AAG CGG AAA GCT GGA ACA TCC ACC CTG ATT GAG TGT GTG ATC Gly Phe Lys Arg Lys Ala Gly Thr Ser Thr Leu Ile Glu Cys Val Ile	240
	65 70 75 80	
40	AAC AAG AAC ACA AAT GTT GCC CAC TGG ACA ACT CCC AGC CTC AAG TGC Asn Lys Asn Thr Asn Val Ala His Trp Thr Thr Pro Ser Leu Lys Cys	288
	85 90 95	
45	ATC AGA GAC CCC TCC CTA GCT CAC TAC AGT CCA GTG CCA ACA GTA GTG Ile Arg Asp Pro Ser Leu Ala His Tyr Ser Pro Val Pro Thr Val Val	336
	100 105 110	
50	ACA CCA AAG GTG ACC TCA CAG CCA GAG AGC CCC TCC CCC TCT GCA AAA Thr Pro Lys Val Thr Ser Gln Pro Glu Ser Pro Ser Pro Ser Ala Lys	384
	115 120 125	
55	GAG CCA GAA GCT TTC TCT CCC AAA TCA GAT ACC GCA ATG ACC ACA GAG Glu Pro Glu Ala Phe Ser Pro Lys Ser Asp Thr Ala Met Thr Thr Glu	432
	130 135 140	
60	ACA GCT ATT ATG CCT GGC TCC AGG CTG ACA CCA TCC CAA ACA ACT TCT Thr Ala Ile Met Pro Gly Ser Arg Leu Thr Pro Ser Gln Thr Thr Ser	480
	145 150 155 160	
65	GCA GGA ACT ACA GGG ACA GGC AGT CAC AAG TCC TCC CGA GCC CCA TCT Ala Gly Thr Thr Gly Thr Gly Ser His Lys Ser Ser Arg Ala Pro Ser	528
	165 170 175	
70	CTT GCA GCA ACA ATG ACC TTG GAG CCT ACA GCC TCC ACC TCC CTC AGG Leu Ala Ala Thr Met Thr Leu Glu Pro Thr Ala Ser Thr Ser Leu Arg	576
	180 185 190	

ATA ACA GAG ATT TCT CCC CAC AGT TCC AAA ATG ACG AAA GTG GCC ATC 624  
 Ile Thr Glu Ile Ser Pro His Ser Ser Lys Met Thr Lys Val Ala Ile  
 195 200 205  
 5 TCT ACA TCG GTC CTC TTG GTT GGT GCA GGG GTT GTG ATG GCT TTC CTG 672  
 Ser Thr Ser Val Leu Leu Val Gly Ala Gly Val Val Met Ala Phe Leu  
 210 215 220  
 10 GCC TGG TAC ATC AAA TCA AGG CAG CCT TCT CAG CCG TGC CGT GTT GAG 720  
 Ala Trp Tyr Ile Lys Ser Arg Gln Pro Ser Gln Pro Cys Arg Val Glu  
 225 230 235 240  
 15 GTG GAA ACC ATG GAA ACA GTA CCA ATG ACT GTG AGG GCC AGC AGC AAG 768  
 Val Glu Thr Met Glu Thr Val Pro Met Thr Val Arg Ala Ser Ser Lys  
 245 250 255  
 GAG GAT GAA GAC ACA GGA GCC TAA 792  
 Glu Asp Glu Asp Thr Gly Ala  
 260

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- 25 (A) LENGTH: 263 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ala Ser Pro Gln Leu Arg Gly Tyr Gly Val Gln Ala Ile Pro Val  
 1 5 10 15  
 35 Leu Leu Leu Leu Leu Leu Leu Leu Leu Pro Leu Arg Val Thr Pro  
 20 25 30  
 40 Gly Thr Thr Cys Pro Pro Pro Val Ser Ile Glu His Ala Asp Ile Arg  
 35 40 45  
 Val Lys Asn Tyr Ser Val Asn Ser Arg Glu Arg Tyr Val Cys Asn Ser  
 50 55 60  
 45 Gly Phe Lys Arg Lys Ala Gly Thr Ser Thr Leu Ile Glu Cys Val Ile  
 65 70 75 80  
 Asn Lys Asn Thr Asn Val Ala His Trp Thr Thr Pro Ser Leu Lys Cys  
 85 90 95  
 50 Ile Arg Asp Pro Ser Leu Ala His Tyr Ser Pro Val Pro Thr Val Val  
 100 105 110  
 55 Thr Pro Lys Val Thr Ser Gln Pro Glu Ser Pro Ser Pro Ser Ala Lys  
 115 120 125  
 Glu Pro Glu Ala Phe Ser Pro Lys Ser Asp Thr Ala Met Thr Thr Glu  
 130 135 140  
 60 Thr Ala Ile Met Pro Gly Ser Arg Leu Thr Pro Ser Gln Thr Thr Ser  
 145 150 155 160



Ala Gly Thr Thr Gly Thr Gly Ser His Lys Ser Ser Arg Ala Pro Ser  
 165 170 175

5 Leu Ala Ala Thr Met Thr Leu Glu Pro Thr Ala Ser Thr Ser Leu Arg  
 180 185 190

Ile Thr Glu Ile Ser Pro His Ser Ser Lys Met Thr Lys Val Ala Ile  
 195 200 205

10 Ser Thr Ser Val Leu Leu Val Gly Ala Gly Val Val Met Ala Phe Leu  
 210 215 220

Ala Trp Tyr Ile Lys Ser Arg Gln Pro Ser Gln Pro Cys Arg Val Glu  
 225 230 235 240

15 Val Glu Thr Met Glu Thr Val Pro Met Thr Val Arg Ala Ser Ser Lys  
 245 250 255

20 Glu Asp Glu Asp Thr Gly Ala  
 260

## (2) INFORMATION FOR SEQ ID NO:3:

25 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 122 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: protein  
 (iii) HYPOTHETICAL: NO

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Asp Tyr Lys Asp Asp Asp Asp Lys Asn Trp Val Asn Val Ile Ser Asp  
 1 5 10 15

40 Leu Lys Lys Ile Glu Asp Leu Ile Gln Ser Met His Ile Asp Ala Thr  
 20 25 30

Leu Tyr Thr Glu Ser Asp Val His Pro Ser Cys Lys Val Thr Ala Met  
 35 40 45

45 Lys Cys Phe Leu Leu Glu Leu Gln Val Ile Ser His Glu Ser Gly Asp  
 50 55 60

50 Thr Asp Ile His Asp Thr Val Glu Asn Leu Ile Ile Leu Ala Asn Asn  
 65 70 75 80

Ile Leu Ser Ser Asn Gly Asn Ile Thr Glu Ser Gly Cys Lys Glu Cys  
 85 90 95

55 Glu Glu Leu Glu Glu Lys Asn Ile Lys Glu Phe Leu Gln Ser Phe Val  
 100 105 110

His Ile Val Gln Met Phe Ile Asn Thr Ser  
 115 120

60

## (2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 24 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GATCTTGGAA CGAGACGACC TGCT

24

## (2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AGCAGGTCGT CTCGTTCCAA

20

## (2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 1534 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

- (A) NAME/KEY: CDS  
(B) LOCATION: 1..753

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

5	CTG CTA CTG CTG CTG CTG CTC CGG CCG CCG GCG ACG CGG GGC ATC ACG Leu Leu Leu Leu Leu Leu Leu Arg Pro Pro Ala Thr Arg Gly Ile Thr	48
10	TGC CCT CCC CCC ATG TCC GTG GAA CAC GCA GAC ATC TGG GTC AAG AGC Cys Pro Pro Pro Met Ser Val Glu His Ala Asp Ile Trp Val Lys Ser	96
15	TAC AGC TTG TAC TCC AGG GAG CGG TAC ATT TGT AAC TCT GGT TTC AAG Tyr Ser Leu Tyr Ser Arg Glu Arg Tyr Ile Cys Asn Ser Gly Phe Lys	144
20	CGT AAA GCC GGC ACG TCC AGC CTG ACG GAG TGC GTG TTG AAC AAG GCC Arg Lys Ala Gly Thr Ser Ser Leu Thr Glu Cys Val Leu Asn Lys Ala	192
25	ACG AAT GTC GCC CAC TGG ACA ACC CCC AGT CTC AAA TGC ATT AGA GAC Thr Asn Val Ala His Trp Thr Thr Pro Ser Leu Lys Cys Ile Arg Asp	240
30	CCT GCC CTG GTT CAC CAA AGG CCA GCG CCA CCC TCC ACA GTA ACG ACG Pro Ala Leu Val His Gln Arg Pro Ala Pro Pro Ser Thr Val Thr Thr	288
35	GCA GGG GTG ACC CCA CAG CCA GAG AGC CTC TCC CCT TCT GGA AAA GAG Ala Gly Val Thr Pro Gln Pro Glu Ser Leu Ser Pro Ser Gly Lys Glu	336
40	CCC GCA GCT TCA TCT CCC AGC TCA AAC AAC ACA GCG GCC ACA ACA GCA Pro Ala Ala Ser Ser Pro Ser Ser Asn Asn Thr Ala Ala Thr Thr Ala	384
45	GCT ATT GTC CCG GGC TCC CAG CTG ATG CCT TCA AAA TCA CCT TCC ACA Ala Ile Val Pro Gly Ser Gln Leu Met Pro Ser Lys Ser Pro Ser Thr	432
50	GGA ACC ACA GAG ATA AGC AGT CAT GAG TCC TCC CAC GGC ACC CCC TCT Gly Thr Thr Glu Ile Ser Ser His Glu Ser Ser His Gly Thr Pro Ser	480
55	CAG ACA ACA GCC AAG ACC TGG GAA CTC ACA GCA TCC GCC TCC CAC CAG Gln Thr Thr Ala Lys Thr Trp Glu Leu Thr Ala Ser Ala Ser His Gln	528
60	CCG CCA GGT GTG TAT CCA CAG GGC CAC AGC GAC ACC ACT GTG GCT ATC Pro Pro Gly Val Tyr Pro Gln Gly His Ser Asp Thr Thr Val Ala Ile	576
65	TCC ACG TCC ACT GTC CTG CTG TGT GGG CTG AGC GCT GTG TCT CTC CTG Ser Thr Ser Thr Val Leu Leu Cys Gly Leu Ser Ala Val Ser Leu Leu	624
70	GCA TGC TAC CTC AAG TCA AGG CAA ACT CCC CCG CTG GCC AGC GTT GAA Ala Cys Tyr Leu Lys Ser Arg Gln Thr Pro Pro Leu Ala Ser Val Glu	672
75	ATG GAA GCC ATG GAG GCT CTG CCG GTG ACT TGG GGG ACC AGC AGC AGA Met Glu Ala Met Glu Ala Leu Pro Val Thr Trp Gly Thr Ser Ser Arg	720

5 GAT GAA GAC TTG GAA AAC TGC TCT CAC CAC CTA TGAAACTCGG GGAAACCAGC 773  
 Asp Glu Asp Leu Glu Asn Cys Ser His His Leu  
 245 250  
 CCAGCTAAGT CCGGAGTGAA GGAGCCTCTC TGCTTTAGCT AAAGACGACT GAGAAGAGGT 833  
 GCAAGGAAGC GGGCTCCAGG AGCAAGCTCA CCAGGCCTCT CAGAAGTCCC AGCAGGATCT 893  
 10 CACGGACTGC CGGGTCGGCG CCTCCTGCGC GAGGGAGCAG GTTCTCCGCA TTCCCATGGG 953  
 CACCACCTGC CTGCCTGTCTG TGCCTTGGAC CCAGGGCCCCA GCTTCCCAGG AGAGACCAAA 1013  
 15 GGCTTCTGAG CAGGATTTTT ATTTTCATTAC AGTGTGAGCT GCCTGGAATA CATGTGGTAA 1073  
 TGAAATAAAA ACCCTGCCCC GAATCTTCCG TCCCTCATCC TAACTTGCAG TTCACAGAGA 1133  
 AAAGTGACAT ACCCAAAGCT CTCTGTCAAT TACAAGGCTT CTCCTGGCGT GGGAGACGTC 1193  
 20 TACAGGGAAG ACACCAGCGT TTGGGCTTCT AACCACCCTG TCTCCAGCTG CTCTGCACAC 1253  
 ATGGACAGGG ACCTGGGAAA GGTGGGAGAG ATGCTGAGCC CAGCGAATCC TCTCCATTGA 1313  
 25 AGGATTCAGG AAGAAGAAAA CTCAACTCAG TGCCATTTTA CGAATATATG CGTTTATATT 1373  
 TATACTTCCT TGTCTATTAT ATCTATACAT TATATATTAT TTGTATTTTG ACATTGTACC 1433  
 TTGTATAAAC AAAATAAAAC ATCTATTTTC AATATTTTTA AAATGCAAAA AAAAAAAAAA 1493  
 30 AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA A 1534

## (2) INFORMATION FOR SEQ ID NO:7:

35 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 251 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear  
 40 (ii) MOLECULE TYPE: protein  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:  
 45 Leu Leu Leu Leu Leu Leu Leu Arg Pro Pro Ala Thr Arg Gly Ile Thr  
 1 5 10 15  
 Cys Pro Pro Pro Met Ser Val Glu His Ala Asp Ile Trp Val Lys Ser  
 20 25 30  
 50 Tyr Ser Leu Tyr Ser Arg Glu Arg Tyr Ile Cys Asn Ser Gly Phe Lys  
 35 40 45  
 Arg Lys Ala Gly Thr Ser Ser Leu Thr Glu Cys Val Leu Asn Lys Ala  
 50 55 60  
 55 Thr Asn Val Ala His Trp Thr Thr Pro Ser Leu Lys Cys Ile Arg Asp  
 65 70 75 80  
 60 Pro Ala Leu Val His Gln Arg Pro Ala Pro Pro Ser Thr Val Thr Thr  
 85 90 95

Ala Gly Val Thr Pro Gln Pro Glu Ser Leu Ser Pro Ser Gly Lys Glu  
100 105 110

5 Pro Ala Ala Ser Ser Pro Ser Ser Asn Asn Thr Ala Ala Thr Thr Ala  
115 120 125

Ala Ile Val Pro Gly Ser Gln Leu Met Pro Ser Lys Ser Pro Ser Thr  
130 135 140

10 Gly Thr Thr Glu Ile Ser Ser His Glu Ser Ser His Gly Thr Pro Ser  
145 150 155 160

Gln Thr Thr Ala Lys Thr Trp Glu Leu Thr Ala Ser Ala Ser His Gln  
165 170 175

15 Pro Pro Gly Val Tyr Pro Gln Gly His Ser Asp Thr Thr Val Ala Ile  
180 185 190

20 Ser Thr Ser Thr Val Leu Leu Cys Gly Leu Ser Ala Val Ser Leu Leu  
195 200 205

Ala Cys Tyr Leu Lys Ser Arg Gln Thr Pro Pro Leu Ala Ser Val Glu  
210 215 220

25 Met Glu Ala Met Glu Ala Leu Pro Val Thr Trp Gly Thr Ser Ser Arg  
225 230 235 240

Asp Glu Asp Leu Glu Asn Cys Ser His His Leu  
245 250

30

## (2) INFORMATION FOR SEQ ID NO:8:

35 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 1641 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

45 (iv) ANTI-SENSE: NO

(ix) FEATURE:  
(A) NAME/KEY: CDS  
(B) LOCATION: 3..839

50

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

55 CG CGC GGC TGC CGG ACC CTC GGT CTC CCG GCG CTG CTA CTG CTG CTG 47  
Arg Gly Cys Arg Thr Leu Gly Leu Pro Ala Leu Leu Leu Leu  
1 5 10 15

60 CTG CTC CGG CCG CCG GCG ACG CGG GAT GCA AGA GAC AGG CTG GCT GTC 95  
Leu Leu Arg Pro Pro Ala Thr Arg Asp Ala Arg Asp Arg Leu Ala Val  
20 25 30

	CTG GCG GGA AGG AGC AGA ATA TCT GAA AGC TTC AAC CAT GAG GTC CAG	143
	Leu Ala Gly Arg Ser Arg Ile Ser Glu Ser Phe Asn His Glu Val Gln	
	35 40 45	
5	ACA CAC GAG GCC TGC GTG AGA CTC AGG ACA ATG GAA AAC TGC CCC CAG	191
	Thr His Glu Ala Cys Val Arg Leu Arg Thr Met Glu Asn Cys Pro Gln	
	50 55 60	
10	TGC CAC CAC CAT CGG ACA AGC AGG CAG CAA GCA GGC ATC ACG TGC CCT	239
	Cys His His His Arg Thr Ser Arg Gln Gln Ala Gly Ile Thr Cys Pro	
	65 70 75	
15	CCC CCC ATG TCC GTG GAA CAC GCA GAC ATC TGG GTC AAG AGC TAC AGC	287
	Pro Pro Met Ser Val Glu His Ala Asp Ile Trp Val Lys Ser Tyr Ser	
	80 85 90 95	
20	TTG TAC TCC AGG GAG CGG TAC ATT TGT AAC TCT GGT TTC AAG CGT AAA	335
	Leu Tyr Ser Arg Glu Arg Tyr Ile Cys Asn Ser Gly Phe Lys Arg Lys	
	100 105 110	
	GCC GGC ACG TCC AGC CTG ACG GAG TGC GTG TTG AAC AAG GCC ACG AAT	383
	Ala Gly Thr Ser Ser Leu Thr Glu Cys Val Leu Asn Lys Ala Thr Asn	
	115 120 125	
25	GTC GCC CAC TGG ACA ACC CCC AGT CTC AAA TGC ATT AAG CCC GCA GCT	431
	Val Ala His Trp Thr Thr Pro Ser Leu Lys Cys Ile Lys Pro Ala Ala	
	130 135 140	
30	TCA TCT CCC AGC TCA AAC AAC ACA GCG GCC ACA ACA GCA GCT ATT GTC	479
	Ser Ser Pro Ser Ser Asn Asn Thr Ala Ala Thr Thr Ala Ala Ile Val	
	145 150 155	
35	CCG GGC TCC CAG CTG ATG CCT TCA AAA TCA CCT TCC ACA GGA ACC ACA	527
	Pro Gly Ser Gln Leu Met Pro Ser Lys Ser Pro Ser Thr Gly Thr Thr	
	160 165 170 175	
40	GAG ATA AGC AGT CAT GAG TCC TCC CAC GGC ACC CCC TCT CAG ACA ACA	575
	Glu Ile Ser Ser His Glu Ser Ser His Gly Thr Pro Ser Gln Thr Thr	
	180 185 190	
	GCC AAG ACC TGG GAA CTC ACA GCA TCC GCC TCC CAC CAG CCG CCA GGT	623
	Ala Lys Thr Trp Glu Leu Thr Ala Ser Ala Ser His Gln Pro Pro Gly	
	195 200 205	
45	GTG TAT CCA CAG GGC CAC AGC GAC ACC ACT GTG GCT ATC TCC ACG TCC	671
	Val Tyr Pro Gln Gly His Ser Asp Thr Thr Val Ala Ile Ser Thr Ser	
	210 215 220	
50	ACT GTC CTG CTG TGT GGG CTG AGC GCT GTG TCT CTC CTG GCA TGC TAC	719
	Thr Val Leu Leu Cys Gly Leu Ser Ala Val Ser Leu Leu Ala Cys Tyr	
	225 230 235	
55	CTC AAG TCA AGG CAA ACT CCC CCG CTG GCC AGC GTT GAA ATG GAA GCC	767
	Leu Lys Ser Arg Gln Thr Pro Pro Leu Ala Ser Val Glu Met Glu Ala	
	240 245 250 255	
60	ATG GAG GCT CTG CCG GTG ACT TGG GGG ACC AGC AGC AGA GAT GAA GAC	815
	Met Glu Ala Leu Pro Val Thr Trp Gly Thr Ser Ser Arg Asp Glu Asp	
	260 265 270	

TTG GAA AAC TGC TCT CAC CAC CTA TGAAACTCAG GGAAACCAGC CCAGCTAAGT 869  
 Leu Glu Asn Cys Ser His His Leu  
 275

5 CCGGAGTGAA GGAGCCTCTC TGCTTTAGCT AAAGACGACT GAGAAGAGGT GCAAGGAAGC 929  
 GGGCTCCAGG AGCAAGCTCA CCAGGCCTCT CAGAAGTCCC AGCAGGATCT CACGGACTGC 989  
 CGGGTCGGCG CCTCCTGCGC GAGGGAGCAG GTTCTCCGCA TTCCCATGGG CACCACCTGC 1049  
 10 CTGCCTGTCTG TGCCTTGGAC CCAGGGCCCA GCTTCCCAGG AGAGACCAAA GGCTTCTGAG 1109  
 CAGGATTTTTT ATTTTCATTAC AGTGTGAGCT GCCTGGAATA CATGTGGTAA TGAAATAAAA 1169  
 15 ACCCTGCCCC GAATCTTCCG TCCCTCATCC TAACTTGCAG TTCACAGAGA AAAGTGACAT 1229  
 ACCCAAAGCT CTCTGTCAAT TACAAGGCTT CTCCTGGCGT GGGAGACGTC TACAGGGAAG 1289  
 ACACCAGCGT TTGGGCTTCT AACCACCCTG TCTCCAGCTG CTCTGCACAC ATGGACAGGG 1349  
 20 ACCTGGGAAA GGTGGGAGAG ATGCTGAGCC CAGCGAATCC TCTCCATTGA AGGATTCAGG 1409  
 AAGAAGAAAA CTCAACTCAG TGCCATTTTA CGAATATATG CGTTTATATT TATACTTCCT 1469  
 25 TGTCTATTAT ATCTATACAT TATATATTAT TTGTATTTTG ACATTGTACC TTGTATAAAC 1529  
 AAAATAAAAC ATCTATTTTC AATAAAAAAA AAAAAAAA AAAAAAAA AAAAAAAA 1589  
 AAAAAAAA AAAAAAAA AAAAAAAA AAAAAAAA AAAAAAAA AA 1641  
 30

## (2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:  
 35 (A) LENGTH: 279 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein  
 40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Arg Gly Cys Arg Thr Leu Gly Leu Pro Ala Leu Leu Leu Leu Leu Leu  
 1 5 10 15  
 45 Leu Arg Pro Pro Ala Thr Arg Asp Ala Arg Asp Arg Leu Ala Val Leu  
 20 25 30  
 50 Ala Gly Arg Ser Arg Ile Ser Glu Ser Phe Asn His Glu Val Gln Thr  
 35 40 45  
 His Glu Ala Cys Val Arg Leu Arg Thr Met Glu Asn Cys Pro Gln Cys  
 50 55  
 His His His Arg Thr Ser Arg Gln Gln Ala Gly Ile Thr Cys Pro Pro  
 65 70 75 80  
 60 Pro Met Ser Val Glu His Ala Asp Ile Trp Val Lys Ser Tyr Ser Leu  
 85 90 95

Tyr Ser Arg Glu Arg Tyr Ile Cys Asn Ser Gly Phe Lys Arg Lys Ala  
 100 105 110  
 5 Gly Thr Ser Ser Leu Thr Glu Cys Val Leu Asn Lys Ala Thr Asn Val  
 115 120 125  
 Ala His Trp Thr Thr Pro Ser Leu Lys Cys Ile Lys Pro Ala Ala Ser  
 130 135 140  
 10 Ser Pro Ser Ser Asn Asn Thr Ala Ala Thr Thr Ala Ala Ile Val Pro  
 145 150 155 160  
 Gly Ser Gln Leu Met Pro Ser Lys Ser Pro Ser Thr Gly Thr Thr Glu  
 165 170 175  
 15 Ile Ser Ser His Glu Ser Ser His Gly Thr Pro Ser Gln Thr Thr Ala  
 180 185 190  
 Lys Thr Trp Glu Leu Thr Ala Ser Ala Ser His Gln Pro Pro Gly Val  
 195 200 205  
 Tyr Pro Gln Gly His Ser Asp Thr Thr Val Ala Ile Ser Thr Ser Thr  
 210 215 220  
 25 Val Leu Leu Cys Gly Leu Ser Ala Val Ser Leu Leu Ala Cys Tyr Leu  
 225 230 235 240  
 Lys Ser Arg Gln Thr Pro Pro Leu Ala Ser Val Glu Met Glu Ala Met  
 245 250 255  
 30 Glu Ala Leu Pro Val Thr Trp Gly Thr Ser Ser Arg Asp Glu Asp Leu  
 260 265 270  
 Glu Asn Cys Ser His His Leu  
 275  
 35

## (2) INFORMATION FOR SEQ ID NO:10:

40 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 1474 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 45 (D) TOPOLOGY: linear

45 (ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

50 (iv) ANTI-SENSE: NO

(ix) FEATURE:

55 (A) NAME/KEY: CDS  
 (B) LOCATION: 83..784

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

60 CCCAGAGCAG CGCTCGCCAC CTCCCCCGG CCTGGGCAGC GCTCGCCCGG GGAGTCCAGC 60



	GGTGTCTCTGT GGAGCTGCCG CC ATG GCC CCG CGG CGG GCG CGC GGC TGC CGG	112
	Met Ala Pro Arg Arg Ala Arg Gly Cys Arg	
	1 5 10	
5	ACC CTC GGT CTC CCG GCG CTG CTA CTG CTG CTG CTC CGG CCG CCG	160
	Thr Leu Gly Leu Pro Ala Leu Leu Leu Leu Leu Leu Arg Pro Pro	
	15 20 25	
10	GCG ACG CGG GGC ATC ACG TGC CCT CCC CCC ATG TCC GTG GAA CAC GCA	208
	Ala Thr Arg Gly Ile Thr Cys Pro Pro Pro Met Ser Val Glu His Ala	
	30 35 40	
15	GAC ATC TGG GTC AAG AGC TAC AGC TTG TAC TCC AGG GAG CGG TAC ATT	256
	Asp Ile Trp Val Lys Ser Tyr Ser Leu Tyr Ser Arg Glu Arg Tyr Ile	
	45 50 55	
20	TGT AAC TCT GGT TTC AAG CGT AAA GCC GGC ACG TCC AGC CTG ACG GAG	304
	Cys Asn Ser Gly Phe Lys Arg Lys Ala Gly Thr Ser Ser Leu Thr Glu	
	60 65 70	
25	TGC GTG TTG AAC AAG GCC ACG AAT GTC GCC CAC TGG ACA ACC CCC AGT	352
	Cys Val Leu Asn Lys Ala Thr Asn Val Ala His Trp Thr Thr Pro Ser	
	75 80 85 90	
30	CTC AAA TGC ATT AAG CCC GCA GCT TCA TCT CCC AGC TCA AAC AAC ACA	400
	Leu Lys Cys Ile Lys Pro Ala Ala Ser Ser Pro Ser Ser Asn Asn Thr	
	95 100 105	
35	GCG GCC ACA ACA GCA GCT ATT GTC CCG GGC TCC CAG CTG ATG CCT TCA	448
	Ala Ala Thr Thr Ala Ala Ile Val Pro Gly Ser Gln Leu Met Pro Ser	
	110 115 120	
40	AAA TCA CCT TCC ACA GGA ACC ACA GAG ATA AGC AGT CAT GAG TCC TCC	496
	Lys Ser Pro Ser Thr Gly Thr Thr Glu Ile Ser Ser His Glu Ser Ser	
	125 130 135	
45	CAC GGC ACC CCC TCT CAG ACA ACA GCC AAG AAC TGG GAA CTC ACA GCA	544
	His Gly Thr Pro Ser Gln Thr Thr Ala Lys Asn Trp Glu Leu Thr Ala	
	140 145 150	
50	TCC GCC TCC CAC CAG CCG CCA GGT GTG TAT CCA CAG GGC CAC AGC GAC	592
	Ser Ala Ser His Gln Pro Pro Gly Val Tyr Pro Gln Gly His Ser Asp	
	155 160 165 170	
55	ACC ACT GTG GCT ATC TCC ACG TCC ACT GTC CTG CTG TGT GGG CTG AGC	640
	Thr Thr Val Ala Ile Ser Thr Ser Thr Val Leu Leu Cys Gly Leu Ser	
	175 180 185	
60	GCT GTG TCT CTC CTG GCA TGC TAC CTC AAG TCA AGG CAA ACT CCC CCG	688
	Ala Val Ser Leu Leu Ala Cys Tyr Leu Lys Ser Arg Gln Thr Pro Pro	
	190 195 200	
65	CTG GCC AGC GTT GAA ATG GAA GCC ATG GAG GCT CTG CCG GTG ACT TGG	736
	Leu Ala Ser Val Glu Met Glu Ala Met Glu Ala Leu Pro Val Thr Trp	
	205 210 215	
70	GGG ACC AGC AGC AGA GAT GAA GAC TTG GAA AAC TGC TCT CAC CAC CTA	784
	Gly Thr Ser Ser Arg Asp Glu Asp Leu Glu Asn Cys Ser His His Leu	
	220 225 230	
75	TGAAACTCGG GGAAACCAGC CCAGCTAAGT CCGGAGTGAA GGAGCCTCTC TGCTTTAGCT	844

AAAGACGACT GAGAAGAGGT GCAAGGAAGC GGGCTCCAGG AGCAAGCTCA CCAGGCCTCT 904  
 CAGAAGTCCC AGCAGGATCT CACGGACTGC CGGGTCGGCG CCTCCTGCGC GAGGGAGCAG 964  
 5 GTTCTCCGCA TTCCCATGGG CACCACCTGC CTGCCTGTCG TGCCTTGGAC CCAGGGCCCA 1024  
 GCTTCCCAGG AGAGACCAAA GGCTTCTGAG CAGGATTTTT ATTTCAATTAC AGTGTGAGCT 1084  
 10 GCCTGGAATA CATGTGGTAA TGAAATAAAA ACCCTGCCCC GAATCTTCCG TCCCTCATCC 1144  
 TAACTTTTCA TACACAGAGA AAAGTGACAT ACCCAAAGCT CTCTGTCAAT TACAAGGCTT 1204  
 CTCCTGGCGT GGGAGACGTC TACAGGGAAG ACACCAGCGT TTGGGCTTCT AACCACCCTG 1264  
 15 TCTCCAGCTG CTCTGCACAC ATGGACAGGG ACCTGGGAAA GGTGGGAGAG ATGCTGAGCC 1324  
 CAGCGAATCC TCTCCATTGA AGGATTCAGG AAGAAGAAAA CTCAACTCAG TGCCATTTTA 1384  
 20 CGAATATATG CGTTTATATT TATACTTCCT TGTCTATTAT ATCTATACAT TATATATTAT 1444  
 TTGTATTTTG ACATTGTACC TTGTATAAAC 1474

25 (2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 234 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

35 Met Ala Pro Arg Arg Ala Arg Gly Cys Arg Thr Leu Gly Leu Pro Ala  
 1 5 10 15  
 40 Leu Leu Leu Leu Leu Leu Leu Arg Pro Pro Ala Thr Arg Gly Ile Thr  
 20 25 30  
 Cys Pro Pro Pro Met Ser Val Glu His Ala Asp Ile Trp Val Lys Ser  
 35 40 45  
 45 Tyr Ser Leu Tyr Ser Arg Glu Arg Tyr Ile Cys Asn Ser Gly Phe Lys  
 50 55 60  
 Arg Lys Ala Gly Thr Ser Ser Leu Thr Glu Cys Val Leu Asn Lys Ala  
 65 70 75 80  
 50 Thr Asn Val Ala His Trp Thr Thr Pro Ser Leu Lys Cys Ile Lys Pro  
 85 90 95  
 55 Ala Ala Ser Ser Pro Ser Ser Asn Asn Thr Ala Ala Thr Thr Ala Ala  
 100 105 110  
 Ile Val Pro Gly Ser Gln Leu Met Pro Ser Lys Ser Pro Ser Thr Gly  
 115 120 125  
 60 Thr Thr Glu Ile Ser Ser His Glu Ser Ser His Gly Thr Pro Ser Gln  
 130 135 140

Thr Thr Ala Lys Asn Trp Glu Leu Thr Ala Ser Ala Ser His Gln Pro  
 145 150 155 160  
 5 Pro Gly Val Tyr Pro Gln Gly His Ser Asp Thr Thr Val Ala Ile Ser  
 165 170 175  
 Thr Ser Thr Val Leu Leu Cys Gly Leu Ser Ala Val Ser Leu Leu Ala  
 180 185 190  
 10 Cys Tyr Leu Lys Ser Arg Gln Thr Pro Pro Leu Ala Ser Val Glu Met  
 195 200 205  
 Glu Ala Met Glu Ala Leu Pro Val Thr Trp Gly Thr Ser Ser Arg Asp  
 210 215 220  
 15 Glu Asp Leu Glu Asn Cys Ser His His Leu  
 225 230  
 20  
 (2) INFORMATION FOR SEQ ID NO:12:  
 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 1510 base pairs  
 25 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULE TYPE: cDNA  
 30 (iii) HYPOTHETICAL: NO  
 (iv) ANTI-SENSE: NO  
 35 (ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 3..356  
 40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:  
 CC AGC TCA AAC AAC ACA GCG GCC ACA ACA GCA GCT ATT GTC CCG GGC 47  
 Ser Ser Asn Asn Thr Ala Ala Thr Thr Ala Ala Ile Val Pro Gly  
 45 1 5 10 15  
 TCC CAG CTG ATG CCT TCA AAA TCA CCT TCC ACA GGA ACC ACA GAG ATA 95  
 Ser Gln Leu Met Pro Ser Lys Ser Pro Ser Thr Gly Thr Thr Glu Ile  
 20 25 30  
 50 AGC AGT CAT GAG TCC TCC CAC GGC ACC CCC TCT CAG ACA ACA GCC AAG 143  
 Ser Ser His Glu Ser Ser His Gly Thr Pro Ser Gln Thr Thr Ala Lys  
 35 40 45  
 55 AAC TGG GAA CTC ACA GCA TCC GCC TCC CAC CAG CCG CCA GGT GTG TAT 191  
 Asn Trp Glu Leu Thr Ala Ser Ala Ser His Gln Pro Pro Gly Val Tyr  
 50 55 60  
 60 CCA CAG GGC CAC AGC GAC ACC ACT GTG GCT ATC TCC ACG TCC ACT GTC 239  
 Pro Gln Gly His Ser Asp Thr Thr Val Ala Ile Ser Thr Ser Thr Val  
 65 70 75

5	CTG CTG TGT GGG CTG AGC GCT GTG TCT CTC CTG GCA TGC TAC CTC AAG	287
	Leu Leu Cys Gly Leu Ser Ala Val Ser Leu Leu Ala Cys Tyr Leu Lys	
	80 85 90 95	
10	TCA AGG GCC TCT GTC TGC TCC TGC CAT CCC CGC AGT GCT GGA CAT ACA	335
	Ser Arg Ala Ser Val Cys Ser Cys His Pro Arg Ser Ala Gly His Thr	
	100 105 110	
15	TGC TCA GTG GGA AGC GTC TGT TGATTTGAGG GCAACCCCT CCTCTTTTCA	386
	Cys Ser Val Gly Ser Val Cys	
	115	
20	AAACCTATGA ACCACCTGCT TTGCAGGCAA ACTCCCCCGC TGGCCAGCGT TGAAATGGAA	446
	GCCATGGAGG CTCTGCCGGT GACTTGGGGG ACCAGCAGCA GAGATGAAGA CTTGGAAAAC	506
	TGCTCTCACC ACCTATGAAA CTCGGGGAAA CCAGCCCAGC TAAGTCCGGA GTGAAGGAGC	566
25	CTCTCTGCTT TAGCTAAAGA CGACTGAGAA GAGGTGCAAG GAAGCGGGCT CCAGGAGCAA	626
	GCTCACCAGG CCTCTCAGAA GTCCCAGCAG GATCTCACGG ACTGCCGGGT CGGCGCCTCC	686
	TGCGCGAGGG AGCAGGTTCT CCGCATTCCC ATGGGCACCA CCTGCCTGCC TGTCGTGCCT	746
30	TGGACCCAGG GCCCAGCTTC CCAGGAGAGA CCAAAGGCTT CTGAGCAGGA TTTTATTTC	806
	ATTACAGTGT GAGCTGCCTG GAATACATGT GGTAATGAAA TAAAAACCCT GCCCCGAATC	866
	TTCCGTCCCT CATCCTAACT TTCAGTTCAC AGAGAAAAGT GACATACCCA AAGCTCTCTG	926
35	TCAATTACAA GGCTTCTCCT GGCCTGGGAG ACGTCTACAG GGAAGACACC AGCGTTTGGG	986
	CTTCTAACCA CCCTGTCTCC AGCTGCTCTG CACACATGGA CAGGGACCTG GGAAAGGTGG	1046
	GAGAGATGCT GAGCCCAGCG AATCCTCTCC ATTGAAGGAT TCAGGAAGAA GAAAACCTCAA	1106
40	CTCAGTGCCA TTTTACGAAT ATATGCGTTT ATATTTATAC TTCCTGTCT ATTATATCTA	1166
	TACATTATAT ATTATTTGTA TTTTGACATT GTACCTTGTA TAAACAAAAT AAAACATCTA	1226
	TTTTCAATAT TTTTAAAATG CATTAAGAGA ATCACCAAGG AGAAATGTTC CACATAAAGG	1286
45	AGGAGAAAGA GTAGGAAGGC AGAGTCCAAG GTGACTGAGT TCAGGTGTTC TTTCCAGAAG	1346
	GAGAAAAAGC CTTGCCTAAA GCTGGCTCCG GTCACAGTTT TGGGGAATTT CCCACAATTC	1406
	CATGTGAGGA GAAGCAGCAT TATCTAATCC ACACAGTGGC AAGTCTGGGC TCAGCTCCCC	1466
50	AGTGGTATAC ACATCGTCTC TTCCCTTCTT CTTCTCTTAC TTTC	1510

(2) INFORMATION FOR SEQ ID NO:13:

- 55 (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 118 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear
- 60 (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

5 Ser Ser Asn Asn Thr Ala Ala Thr Thr Ala Ala Ile Val Pro Gly Ser  
 1 5 10 15  
 Gln Leu Met Pro Ser Lys Ser Pro Ser Thr Gly Thr Thr Glu Ile Ser  
 20 25 30  
 10 Ser His Glu Ser Ser His Gly Thr Pro Ser Gln Thr Thr Ala Lys Asn  
 35 40 45  
 Trp Glu Leu Thr Ala Ser Ala Ser His Gln Pro Pro Gly Val Tyr Pro  
 50 55 60  
 15 Gln Gly His Ser Asp Thr Thr Val Ala Ile Ser Thr Ser Thr Val Leu  
 65 70 75 80  
 Leu Cys Gly Leu Ser Ala Val Ser Leu Leu Ala Cys Tyr Leu Lys Ser  
 85 90 95  
 20 Arg Ala Ser Val Cys Ser Cys His Pro Arg Ser Ala Gly His Thr Cys  
 100 105 110  
 25 Ser Val Gly Ser Val Cys  
 115

## (2) INFORMATION FOR SEQ ID NO:14:

30 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 225 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 35 (D) TOPOLOGY: linear  
 (ii) MOLECULE TYPE: Protein  
 (iii) HYPOTHETICAL: YES  
 40 (iv) ANTI-SENSE: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

45 Thr Arg Gly Ile Thr Cys Pro Pro Pro Met Ser Val Glu His Ala Asp  
 1 5 10 15  
 Ile Trp Val Lys Ser Tyr Ser Leu Tyr Ser Arg Glu Arg Tyr Ile Cys  
 20 25 30  
 50 Asn Ser Gly Phe Lys Arg Lys Ala Gly Thr Ser Ser Leu Thr Glu Cys  
 35 40 45  
 55 Val Leu Asn Lys Ala Thr Asn Val Ala His Trp Thr Thr Pro Ser Leu  
 50 55 60  
 Lys Cys Ile Arg Asp Pro Ala Leu Val His Gln Arg Pro Ala Pro Pro  
 65 70 75 80  
 60 Ser Thr Val Thr Thr Ala Gly Val Thr Pro Gln Pro Glu Ser Leu Ser  
 85 90 95

Pro Ser Gly Lys Glu Pro Ala Ala Ser Ser Pro Ser Ser Asn Asn Thr  
 100 105 110

5 Ala Ala Thr Thr Ala Ala Ile Val Pro Gly Ser Gln Leu Met Pro Ser  
 115 120 125

Lys Ser Pro Ser Thr Gly Thr Thr Glu Ile Ser Ser His Glu Ser Ser  
 130 135 140

10 His Gly Thr Pro Ser Gln Thr Thr Ala Lys Thr Trp Glu Leu Thr Ala  
 145 150 155 160

15 Ser Ala Ser His Gln Pro Pro Gly Val Tyr Pro Gln Gly His Ser Asp  
 165 170 175

Thr Thr Val Ala Ile Ser Thr Ser Thr Val Leu Leu Cys Gly Leu Ser  
 180 185 190

20 Ala Val Ser Leu Leu Ala Cys Tyr Leu Lys Ser Arg Ala Ser Val Cys  
 195 200 205

Ser Cys His Pro Arg Ser Ala Gly His Thr Cys Ser Val Gly Ser Val  
 210 215 220

25 Cys  
 225

30 (2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 267 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Protein

40 (iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Met Ala Pro Arg Arg Ala Arg Gly Cys Arg Thr Leu Gly Leu Pro Ala  
 1 5 10 15

50 Leu Leu Leu Leu Leu Leu Arg Pro Pro Ala Thr Arg Gly Ile Thr  
 20 25 30

Cys Pro Pro Pro Met Ser Val Glu His Ala Asp Ile Trp Val Lys Ser  
 35 40 45

55 Tyr Ser Leu Tyr Ser Arg Glu Arg Tyr Ile Cys Asn Ser Gly Phe Lys  
 50 55 60

60 Arg Lys Ala Gly Thr Ser Ser Leu Thr Glu Cys Val Leu Asn Lys Ala  
 65 70 75 80

	Thr	Asn	Val	Ala	His	Trp	Thr	Thr	Pro	Ser	Leu	Lys	Cys	Ile	Arg	Asp	85	90	95
5	Pro	Ala	Leu	Val	His	Gln	Arg	Pro	Ala	Pro	Pro	Ser	Thr	Val	Thr	Thr	100	105	110
	Ala	Gly	Val	Thr	Pro	Gln	Pro	Glu	Ser	Leu	Ser	Pro	Ser	Gly	Lys	Glu	115	120	125
10	Pro	Ala	Ala	Ser	Ser	Pro	Ser	Ser	Asn	Asn	Thr	Ala	Ala	Thr	Thr	Ala	130	135	140
	Ala	Ile	Val	Pro	Gly	Ser	Gln	Leu	Met	Pro	Ser	Lys	Ser	Pro	Ser	Thr	145	150	155
15	Gly	Thr	Thr	Glu	Ile	Ser	Ser	His	Glu	Ser	Ser	His	Gly	Thr	Pro	Ser	165	170	175
20	Gln	Thr	Thr	Ala	Lys	Thr	Trp	Glu	Leu	Thr	Ala	Ser	Val	Ser	His	Gln	180	185	190
	Pro	Thr	Gly	Val	Phe	Pro	Gln	Gly	His	Ser	Asp	Thr	Thr	Val	Ala	Ile	195	200	205
25	Ser	Thr	Ser	Thr	Val	Leu	Leu	Cys	Gly	Leu	Ser	Ala	Val	Ser	Leu	Leu	210	215	220
	Ala	Cys	Tyr	Leu	Lys	Ser	Arg	Gln	Thr	Pro	Pro	Leu	Ala	Ser	Val	Glu	225	230	235
30	Met	Glu	Ala	Met	Glu	Ala	Leu	Pro	Val	Thr	Trp	Gly	Thr	Ser	Ser	Arg	245	250	255
35	Asp	Glu	Asp	Leu	Glu	Asn	Cys	Ser	His	His	Leu						260	265	

**CLAIMS**

We claim:

1. An isolated DNA encoding an IL-15 receptor (IL-15R), wherein the DNA is selected from the group consisting of:
  - (a) a DNA having a nucleotide sequence as set forth in SEQ ID NO:1, nucleotides 91 through 789;
  - (b) a DNA having a nucleotide sequence as set forth in SEQ ID NO:6, nucleotides 34 through 753;
  - (c) DNAs that hybridize to the DNA sequences of (a) or (b) or their complementary strands under conditions of high stringency, and which encode polypeptides capable of binding IL-15; and
  - (d) DNAs that, due to degeneracy of the genetic code, encode polypeptides encoded by any of the foregoing DNAs.
2. An isolated DNA according to claim 1 that encodes a human IL-15R.
3. An isolated DNA according to claim 1 that encodes a soluble IL-15R.
4. An isolated DNA according to claim 3 wherein the DNA is selected from the group consisting of:
  - (a) a DNA having a nucleotide sequence as set forth in SEQ ID NO:1, nucleotides 91 through 612;
  - (b) a DNA having a nucleotide sequence as set forth in SEQ ID NO:6, nucleotides 34 through 567;
  - (c) a DNA that encodes a fragment of a peptide encoded by the sequences of (a) or (b), which fragment is capable of binding IL-15;
  - (d) DNAs that hybridize to the DNA sequences of (a), (b) or (c) or their complementary strands under conditions of high stringency, and which encode a polypeptide capable of binding IL-15; and
  - (e) DNAs that, due to degeneracy of the genetic code, encode a polypeptide encoded by any of the foregoing DNAs.
5. An isolated DNA sequence according to claim 3 that encodes a human IL-15R.



6. An isolated DNA according to claim 1, encoding a modified IL-15R polypeptide having one or more changes in a primary amino acid sequence, which changes are selected from the group consisting of: inactivated N-linked glycosylation sites; modified KEX2 protease cleavage sites; deleted cysteine residues; and conservative amino acid substitutions, wherein the modified polypeptide binds IL-15.

7. A recombinant expression vector comprising a DNA according to claim 1.

8. A recombinant expression vector comprising a DNA according to claim 4.

9. A recombinant expression vector comprising a DNA according to claim 5.

10. A process for preparing an IL-15 receptor (IL-15R), comprising culturing a host cell transformed or transfected with a recombinant expression vector according to claim 7 under conditions promoting expression, and recovering a polypeptide from the culture, wherein the polypeptide is capable of binding IL-15.

11. A process for preparing an IL-15 receptor (IL-15R), comprising culturing a host cell transformed or transfected with a recombinant expression vector according to claim 8 under conditions promoting expression, and recovering a polypeptide from the culture, wherein the polypeptide is capable of binding IL-15.

12. A process for preparing an IL-15 receptor (IL-15R), comprising culturing a host cell transformed or transfected with a recombinant expression vector according to claim 9 under conditions promoting expression, and recovering a polypeptide from the culture, wherein the polypeptide is capable of binding IL-15.

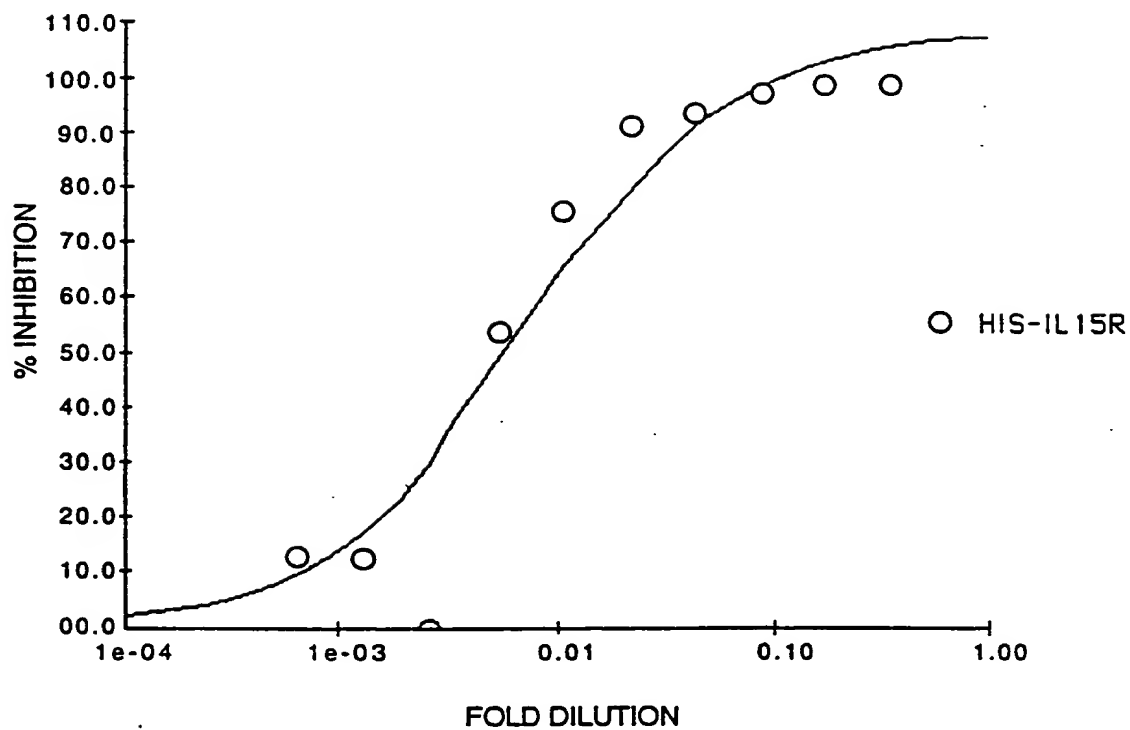
13. An isolated IL-15 receptor (IL-15R) encoded by a DNA sequence according to claim 1.

14. An IL-15 receptor (IL-15R) according to claim 13 which is a soluble IL-15R.

15. An IL-15 receptor (IL-15R) according to claim 13, comprising a polypeptide selected from the group consisting of:

- (a) a polypeptide having an amino acid sequence as set forth in SEQ ID NO:2, having an amino terminus selected from the group consisting of amino acid 31, amino acid 34 and amino acid 35 of SEQ ID NO:2, and a carboxy

- terminus selected from the group consisting of amino acid 204 and amino acid 205 of SEQ ID NO:2:
- (b) a polypeptide having an amino acid sequence as set forth in SEQ ID NO:6, having an amino terminus selected from the group consisting of amino acid 12, amino acid 15 and amino acid 16 of SEQ ID NO:6, and a carboxy terminus selected from the group consisting of an amino acid between amino acid 78 and amino acid 189 of SEQ ID NO:6; and
  - (c) a fragment of a polypeptide having an amino acid sequence of the polypeptide of (a) or (b), which fragment is capable of binding IL-15.
17. Antibodies immunoreactive with mammalian IL-15 receptors.
18. An antibody according to claim 16 which is a monoclonal antibody.
19. An antibody according to claim 17 which is a blocking monoclonal antibody.

**Figure 1**

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10.

9.

## Figure 2

# INTERNATIONAL SEARCH REPORT

I. National application No.  
PCT/US95/05585

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C07K 14/715, 16/24; C07H 21/04; C12N 15/12, 15/63  
US CL : 530/350, 351, 387.9; 536/23.5; 435/69.1, 172.1, 320.1

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/350, 351, 387.9; 536/23.5; 435/69.1, 172.1, 320.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  
Cytokine Facts Book (Academic Press, 1994)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG

search terms: Interleukin 15 (no synonyms).

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication where appropriate, of the relevant passages	Relevant to claim No.
Y --- A	J. Cellular Biochemistry, Supplement, Volume 18D, issued 1994, J.G. Giri et al., "Biochemical characterization of a novel cytokine IL-15 and its receptor", abstract V561, page 400, see entire abstract.	1-16 ----- 17-19
Y,P ---- A,P	Science, Vol. 264, issued 13 May 1994, K.H. Grabstein et al., "Cloning of a T-cell growth factor that interacts with the $\beta$ chain of the interleukin-2 receptor", pages 965-968, see entire document.	1-16 ----- 17-19
Y --- A	EMBO Journal, Volume 13, No. 12, issued 1994, J.G. Giri et al., "Utilization of the beta chain and gamma chain of the IL-2 receptor by the novel cytokin IL-15", pages 2822-2830, see entire document.	1-16 ----- 17-19

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z*	document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means		
*P* document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

19 JUNE 1995

Date of mailing of the international search report

10 JUL 1995

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# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US95/05585

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P ---- Y,P	J. Exp. Med., Volume 180, issued October 1994, W.E. Carson et al., "Interleukin(IL) 15 is a novel cytokine that activates human natural killer cells via components of the IL-2 receptor", pages 1395-1403, see entire document.	17-19 ----- 1-16
A	Scand. J. Immunol., Volume 38, issued 1993, C. Lindqvist et al., "Expression of human IL-2 receptor $\alpha$ - and $\beta$ -chains using the baculovirus expression system", pages 267-272, see entire document.	1-16
X --- A	J. Immunol., Volume 149, No. 8, issued 15 October 1992, R.J. Kreitman et al., "Mik- $\beta$ (Fv)-PE40, a recombinant immunotoxin cytotoxic toward cells bearing the $\beta$ -chain of the IL-2 receptor", pages 2810-2815, see entire document.	17-19 ----- 1-16